

00441594-1101-9

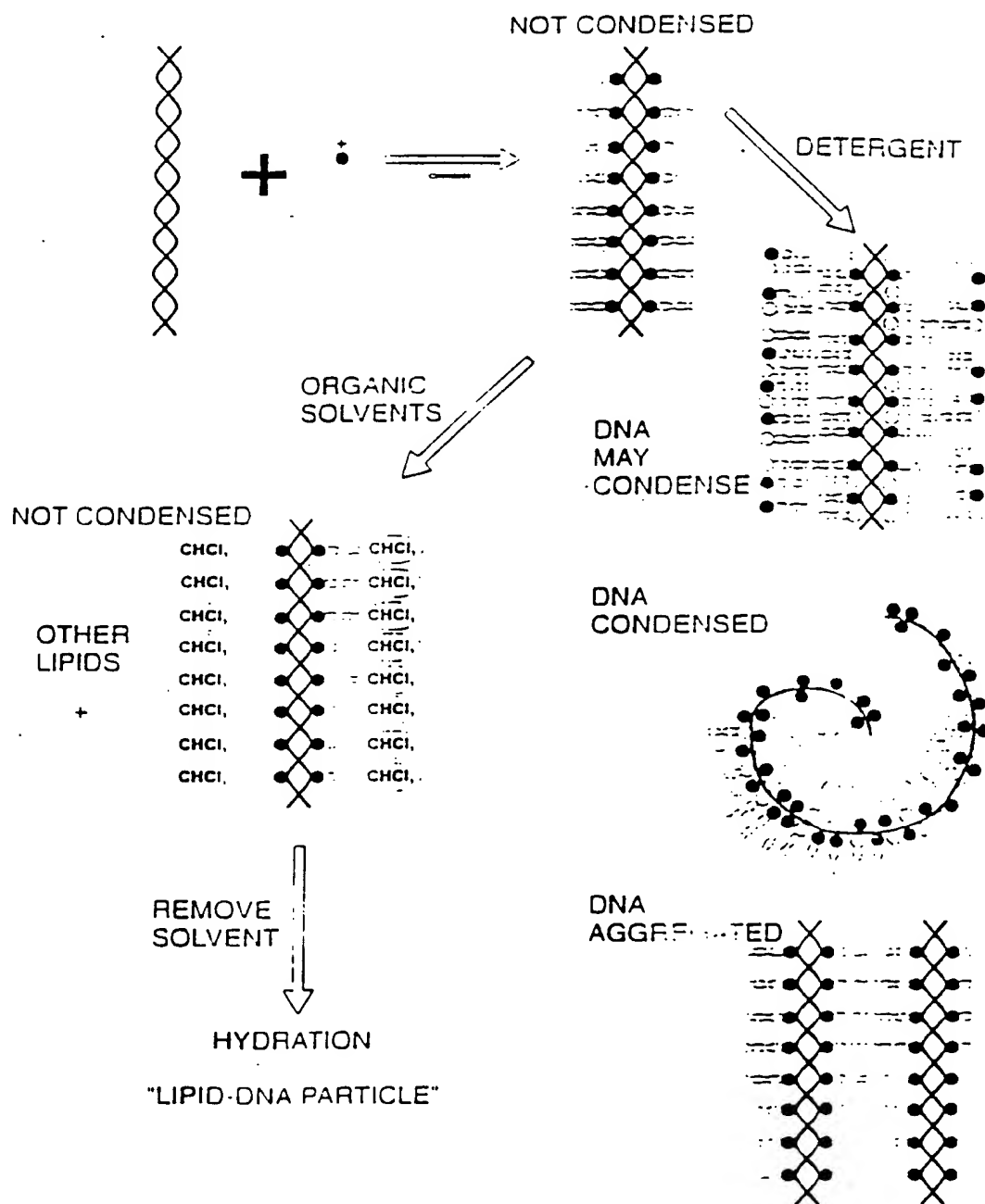


Figure 1

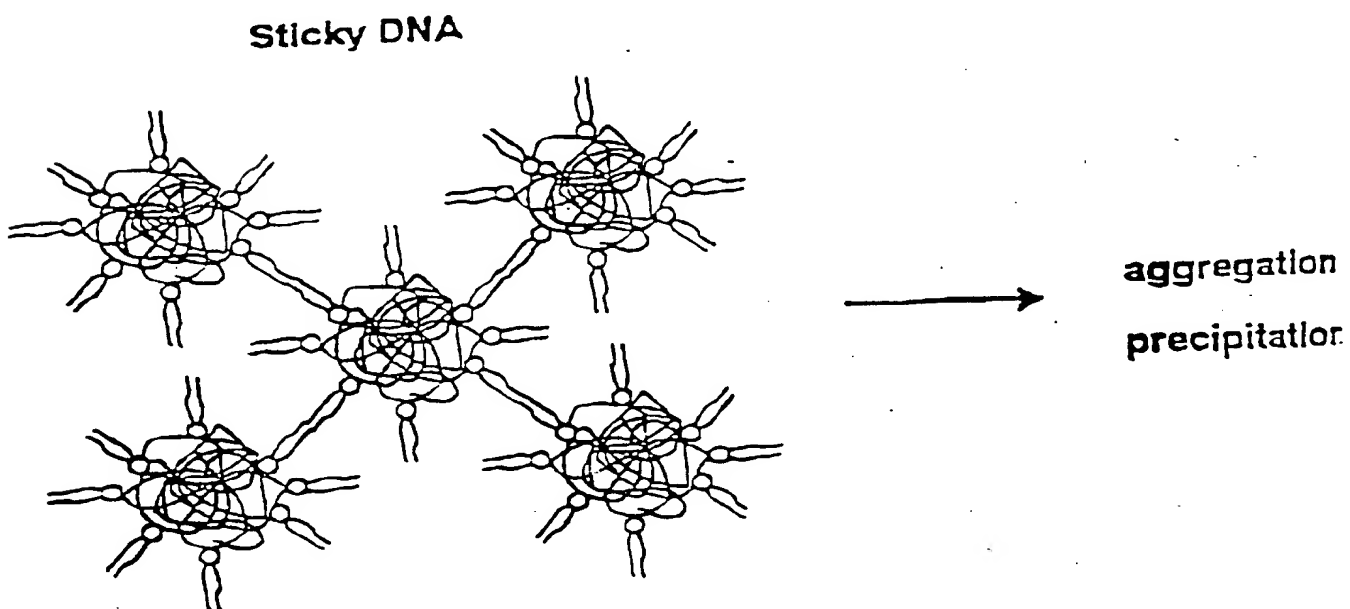
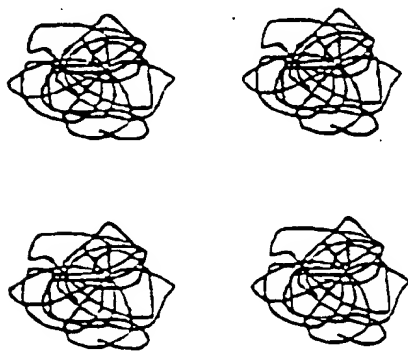


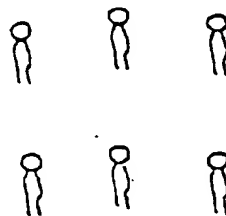
Figure 2

Negatively Charged Plasmid

Positively Charged Lipid

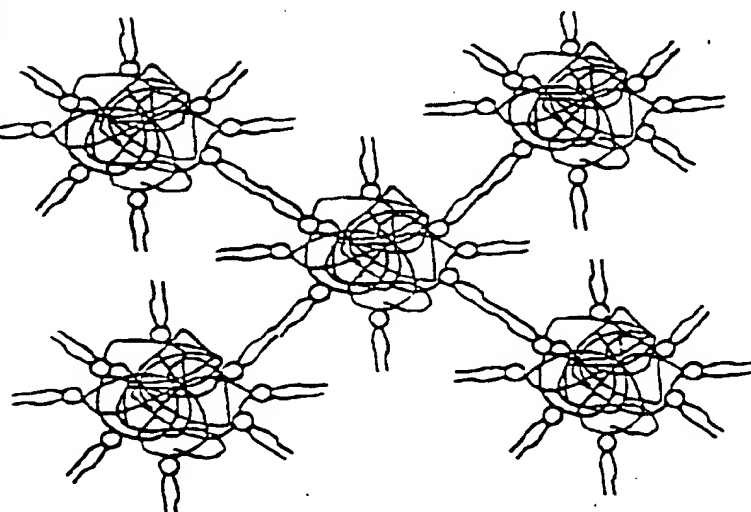


+

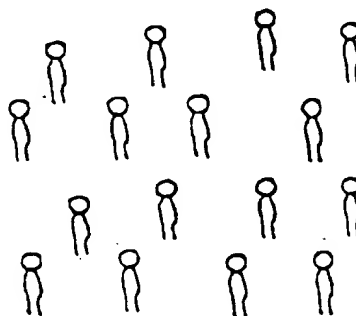


Sticky DNA

Excess Lipids



+



Plasmid

Encapsulated Within a Lipid Bilayer

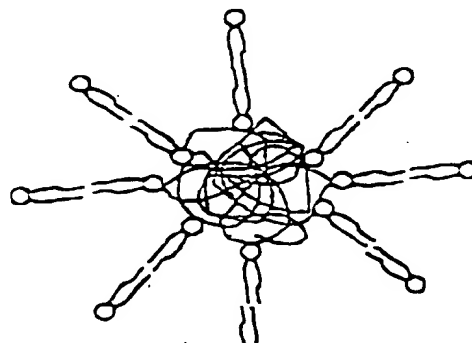
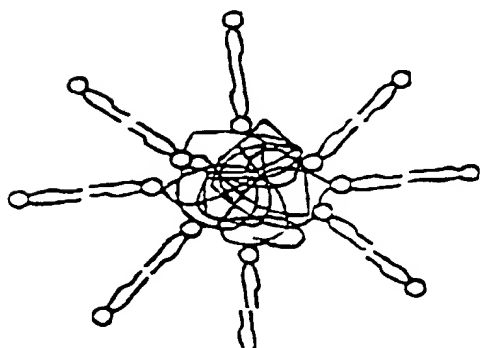


Figure 3

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Figure 1
Recovery of DNA After Extrusion
(20 mg total lipid)

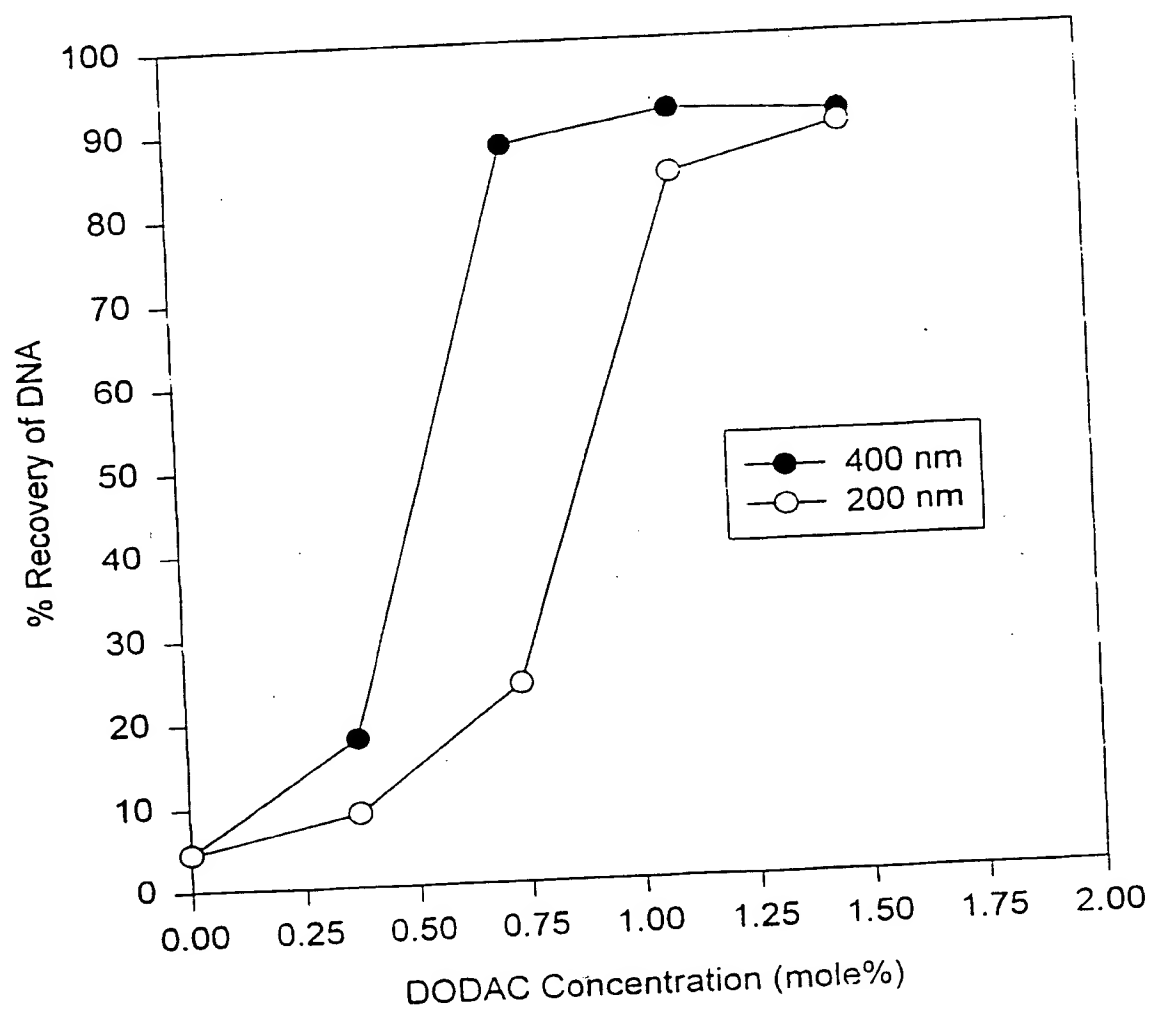


Figure 4

Figure 2
Anion Exchange Chromatography
(20 mg total lipid)

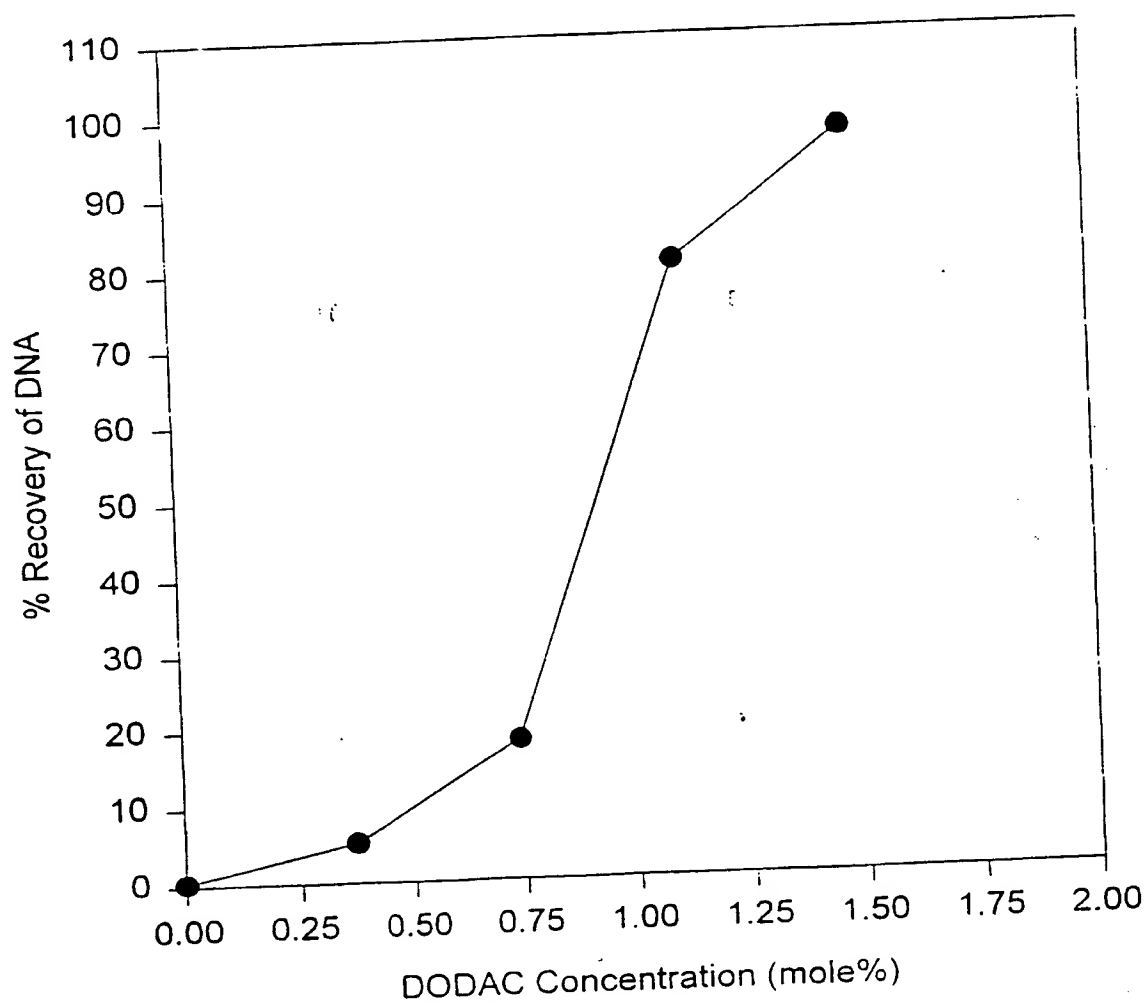


Figure 5

Recovery of Lipid After Extrusion
POPC:DODAC:PEG-Cer(C20), 20 mg

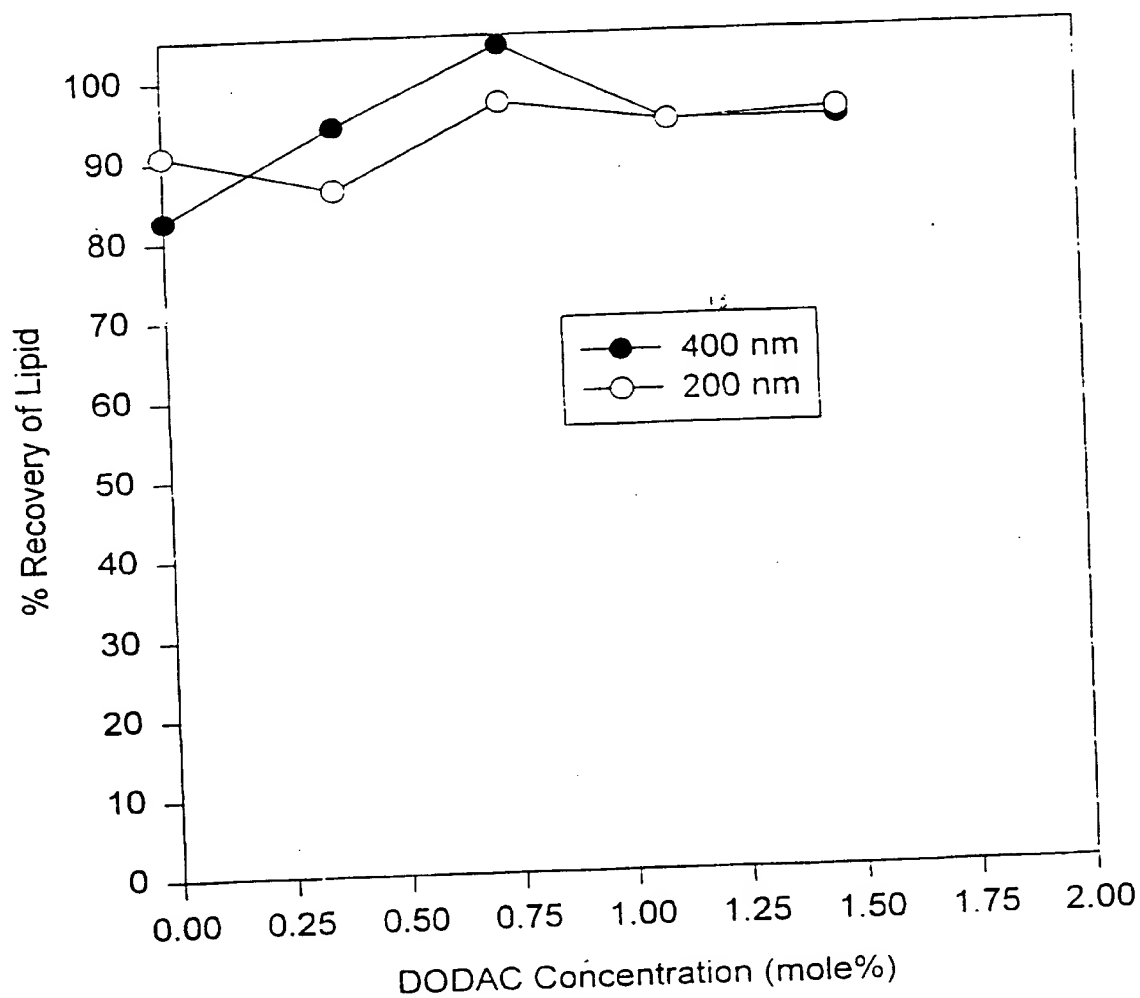


Figure 6

Anion Exchange Chromatography
POPC:DODAC:PEG-Cer(C20), 20 mg

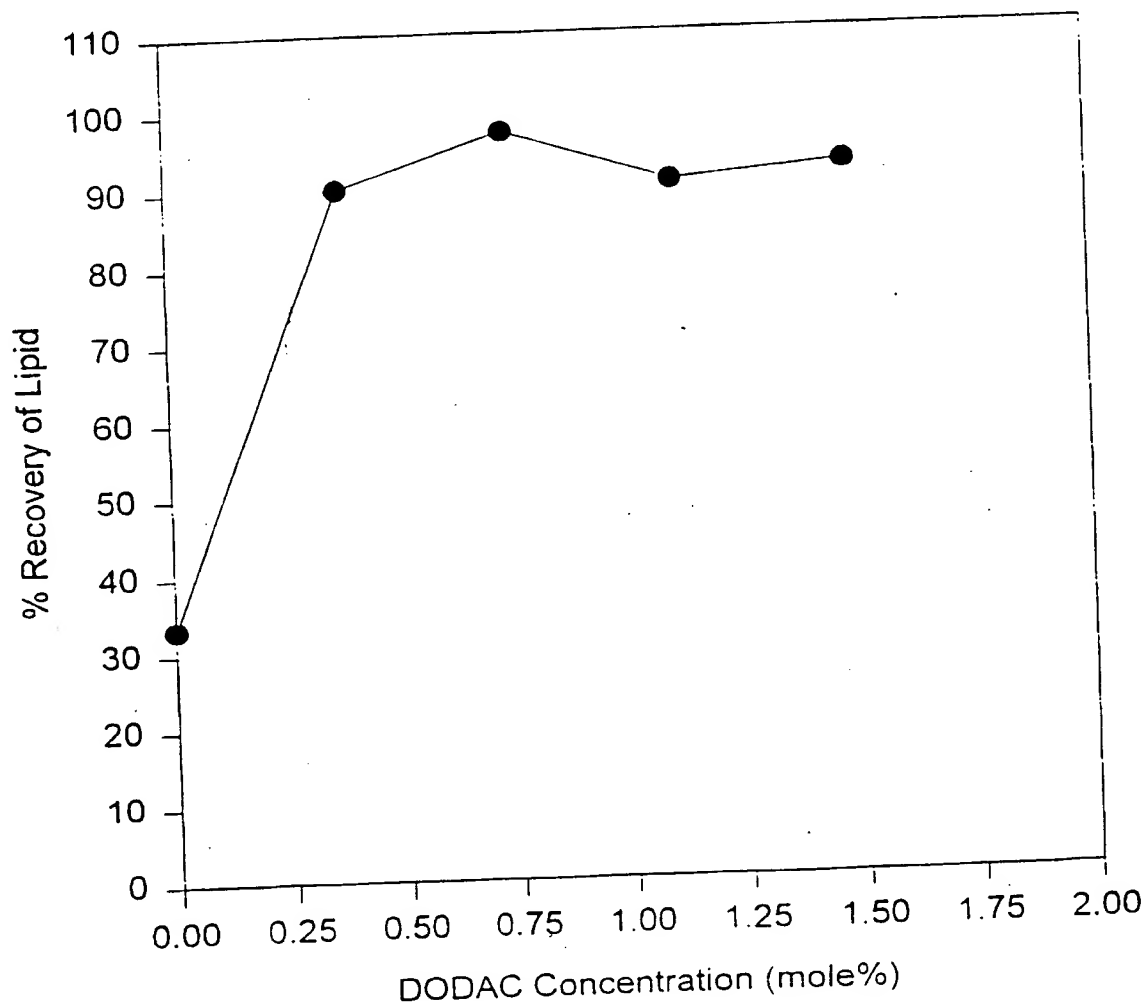


Figure 7

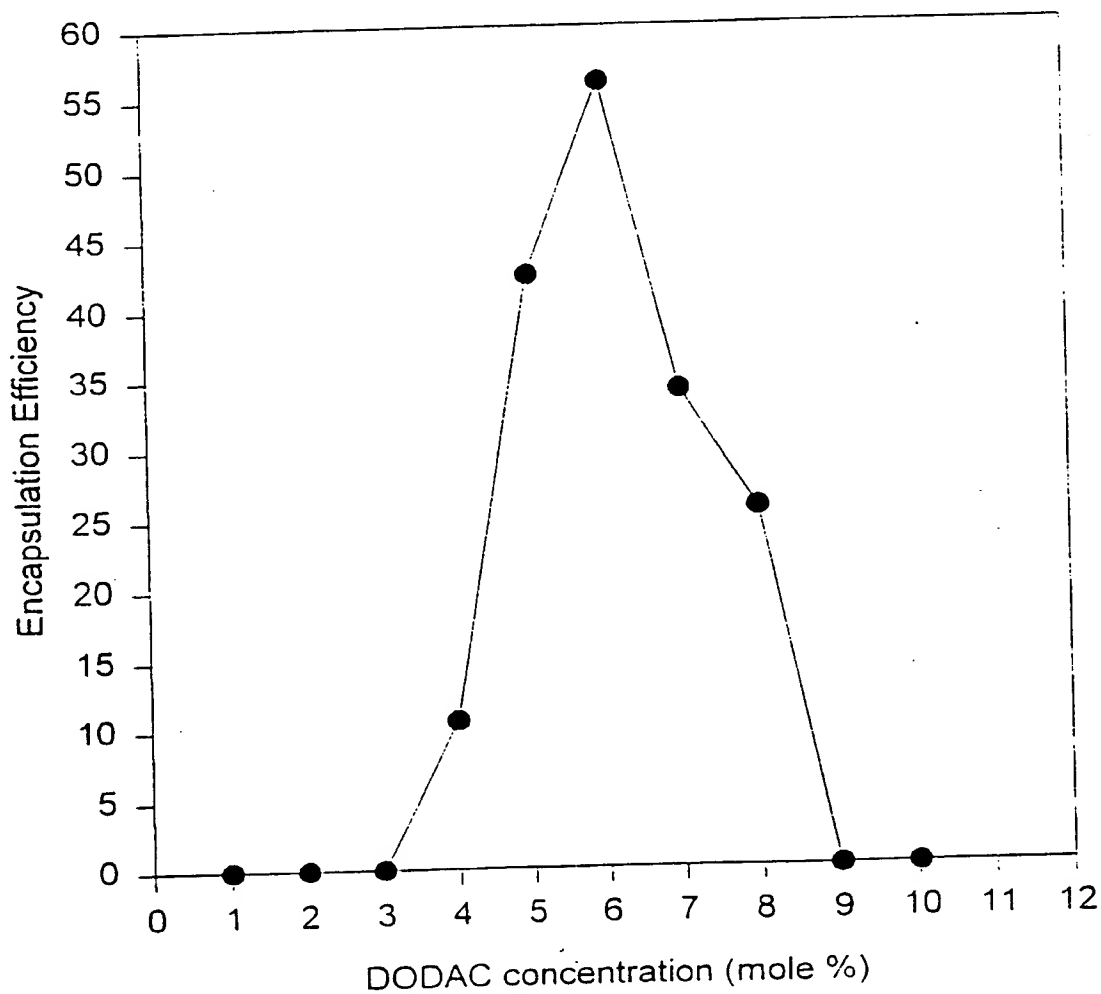


Figure 8

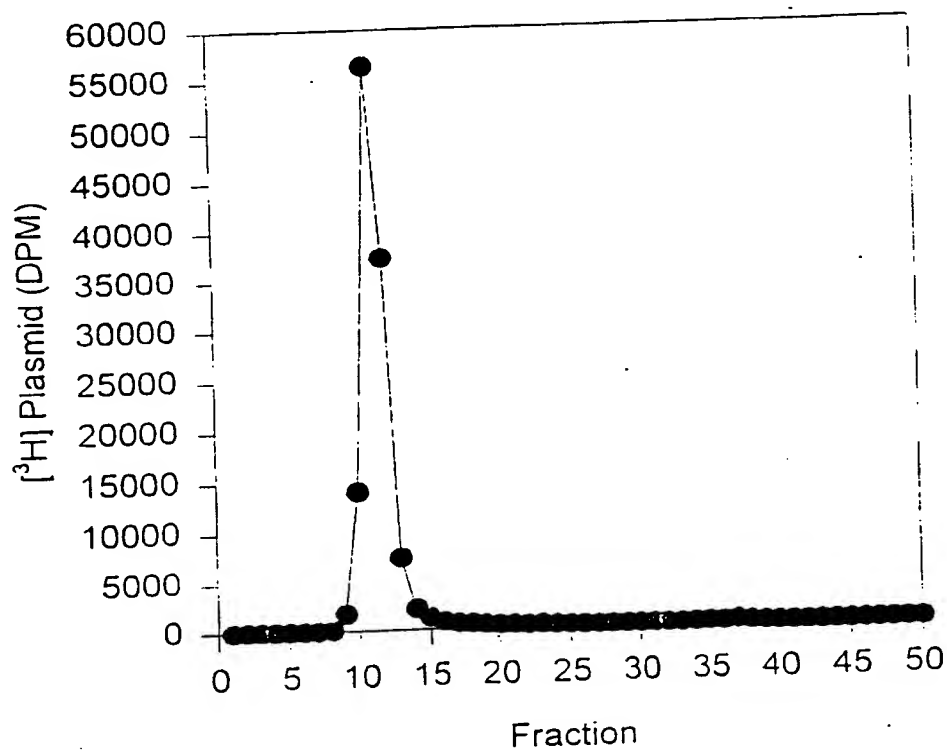


Figure 9A

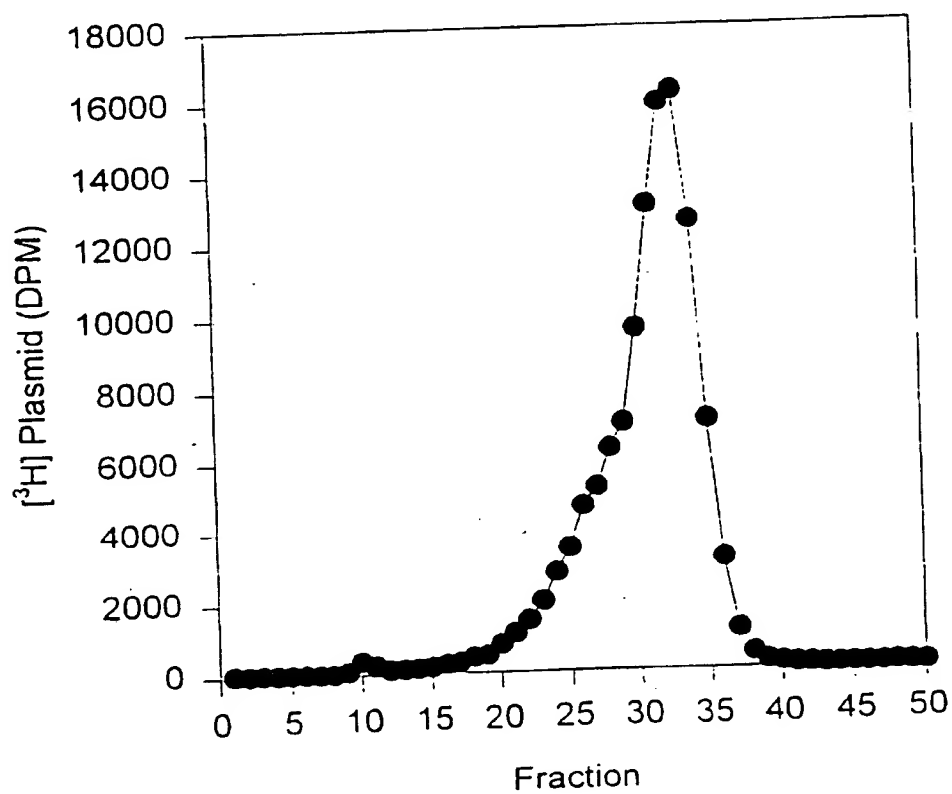


Figure 9B

05/31/96

02:35

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INEX

031/043

Recovery of ^3H DNA and ^{14}C Lipid
After Incubation in Mouse Serum
POPC:DODAC:PEG-Cer(C20)

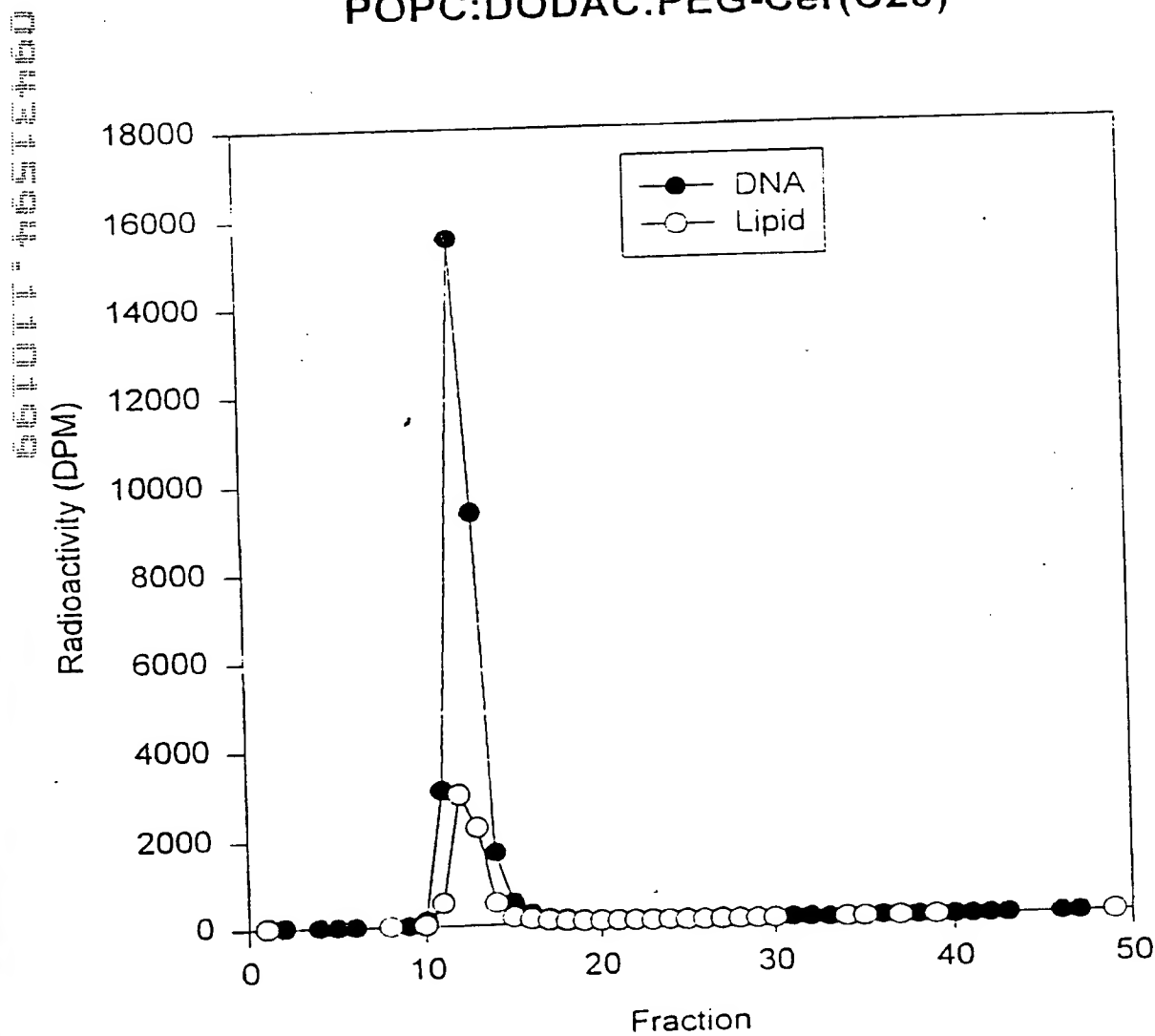


Figure 10

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INEX

032/043

604 264 9959

Figure 11A

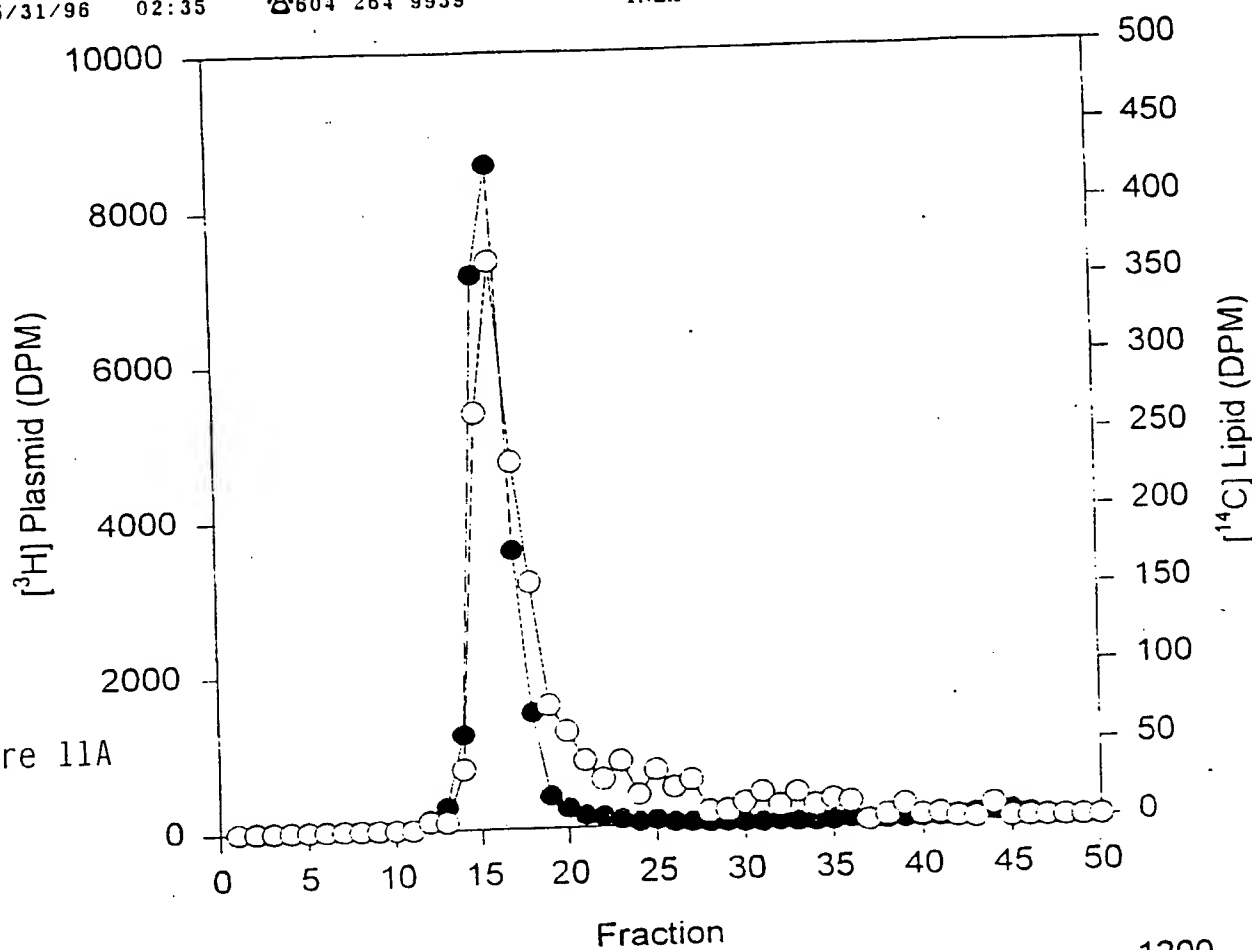
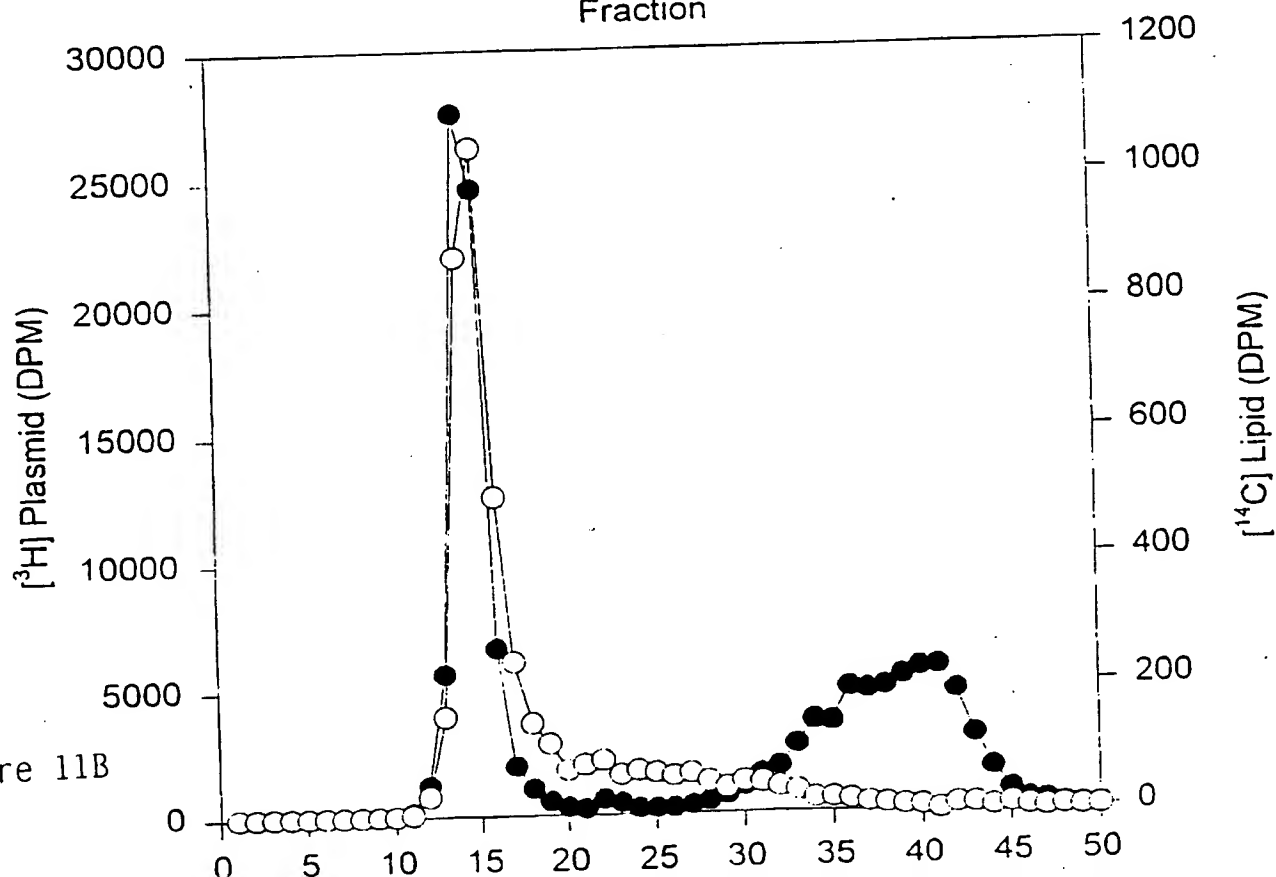


Figure 11B



05/31/96

02:36

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INEX

033/043

A

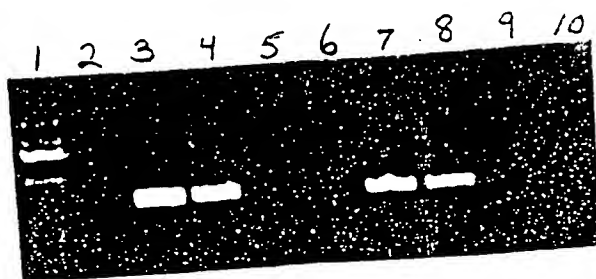


Figure 12A

B

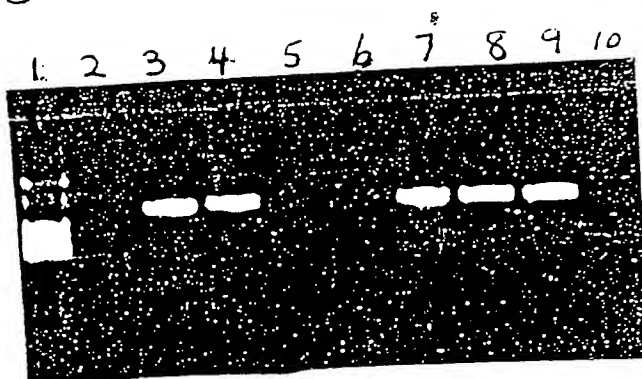


Figure 12B

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INEX

034/043

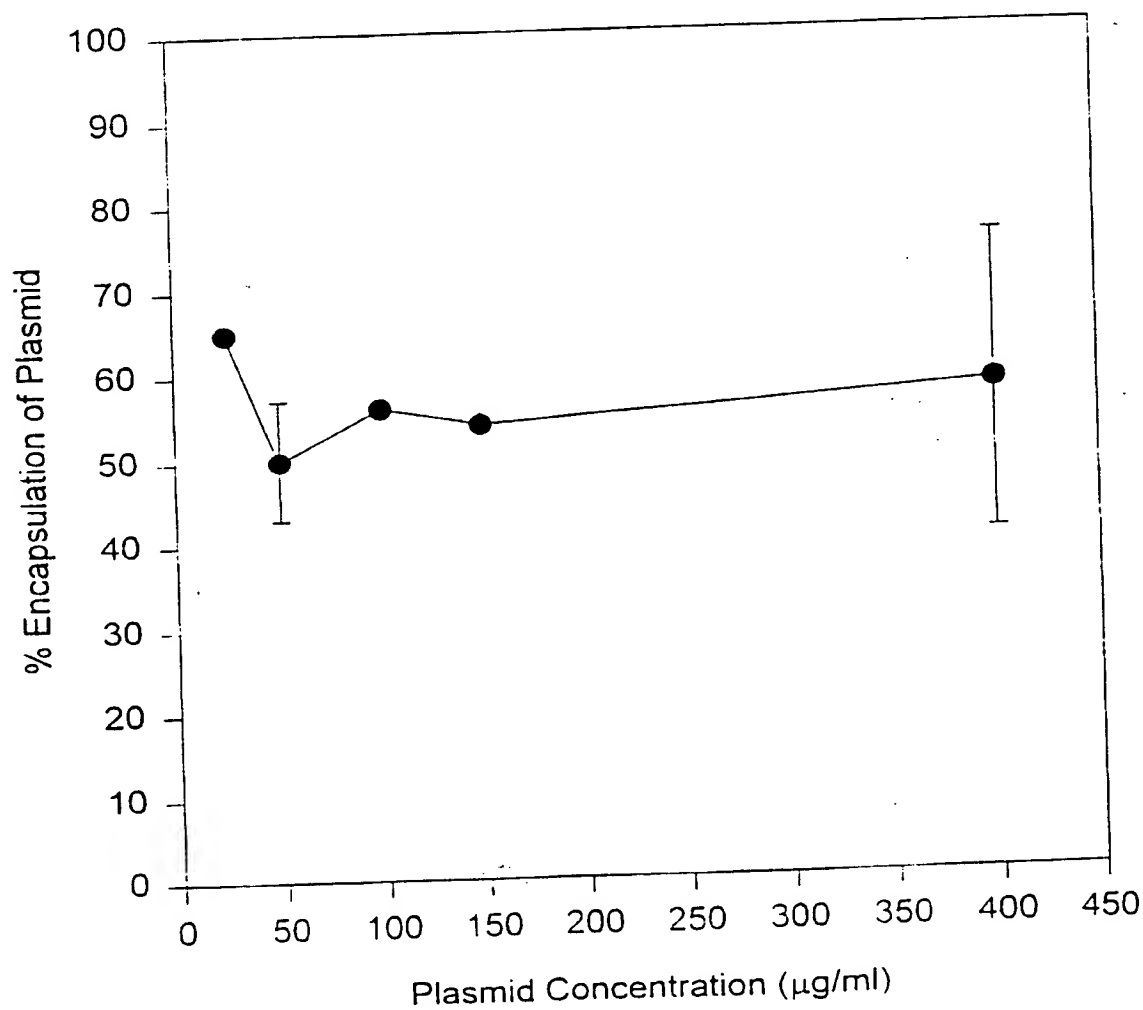


Figure 13

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INEX

035/043

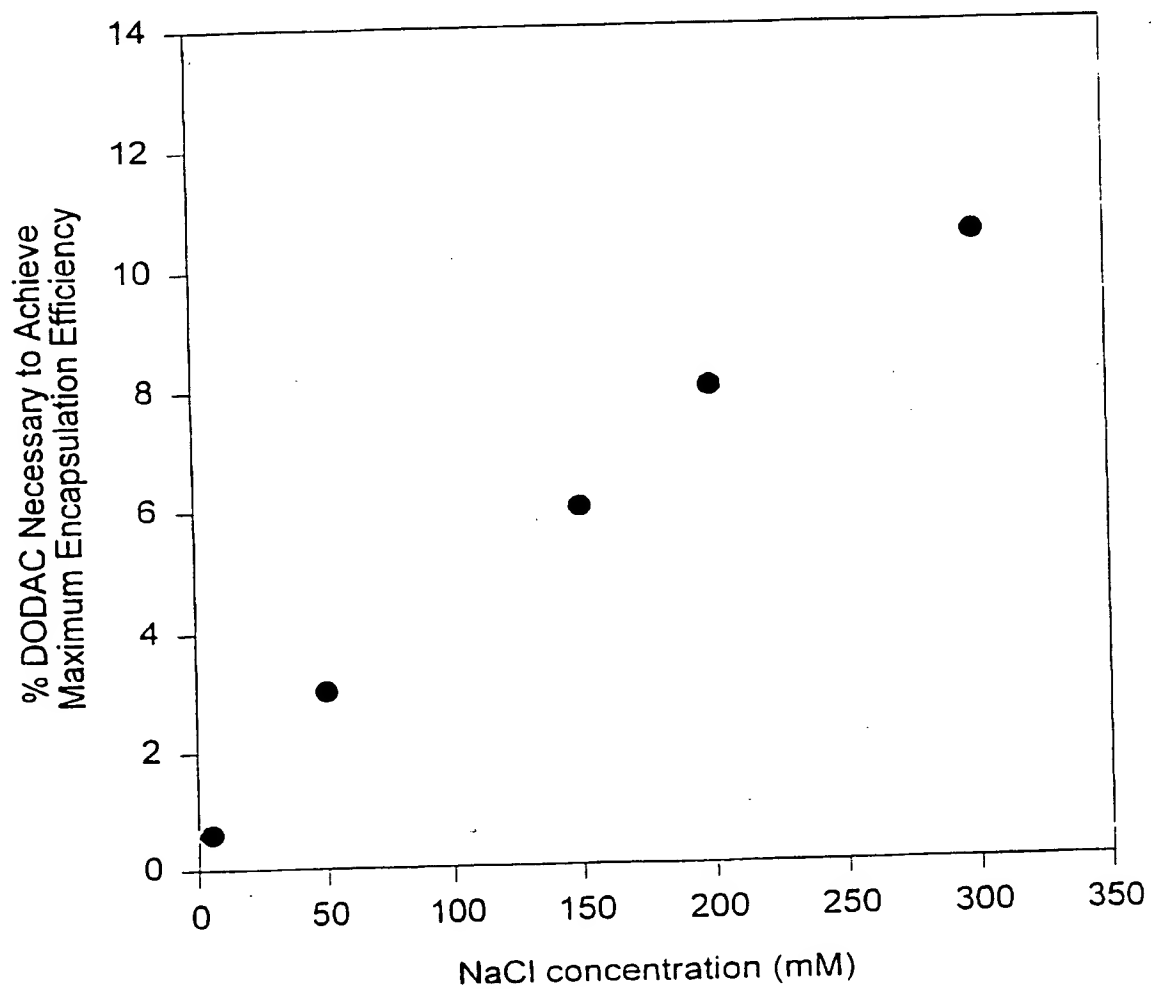


Figure 14

05/31/96

02:37

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INEX

036/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment
Stored Data File a:\PEGC20.06

VOLUME-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 61.6 nm

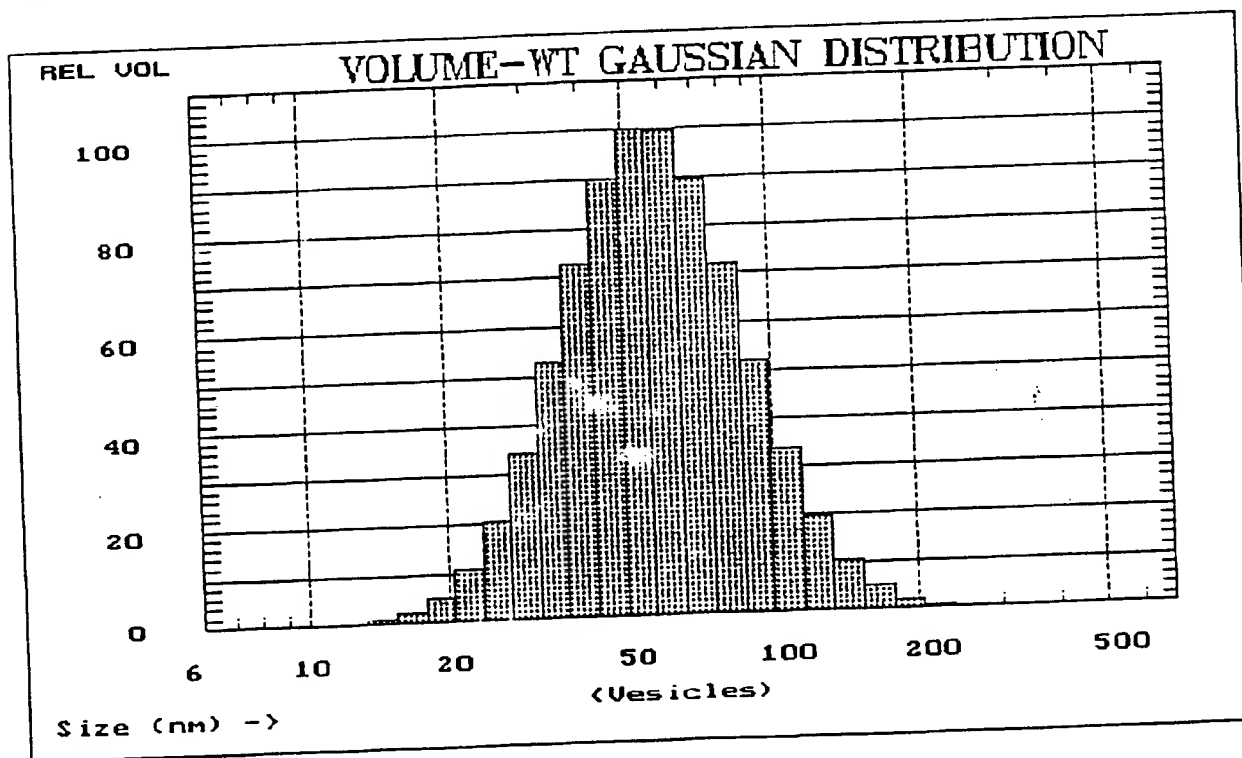
Std. Deviation = 27.0 nm (43.9 %)

Coeff. of Var'n = 0.439

Chi Squared = 0.347

Baseline Adj. = 0.000 %

Mean Diff. Coeff. = 7.54E-08 cm²/s



Cumulative Results:

25 % of distribution < 38.73 nm
50 % of distribution < 52.05 nm
75 % of distribution < 69.91 nm
90 % of distribution < 91.36 nm
99 % of distribution < 142.99 nm

Figure 15

Run Time = 1 Hr 43 Min 26 Sec
Count Rate = 303 KHz
Channel #1 = 2827.4 K
Channel Width = 8.0 uSec

Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

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INEX

037/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment
Stored Data File a:\PEGC20.06

NUMBER-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 32.8 nm

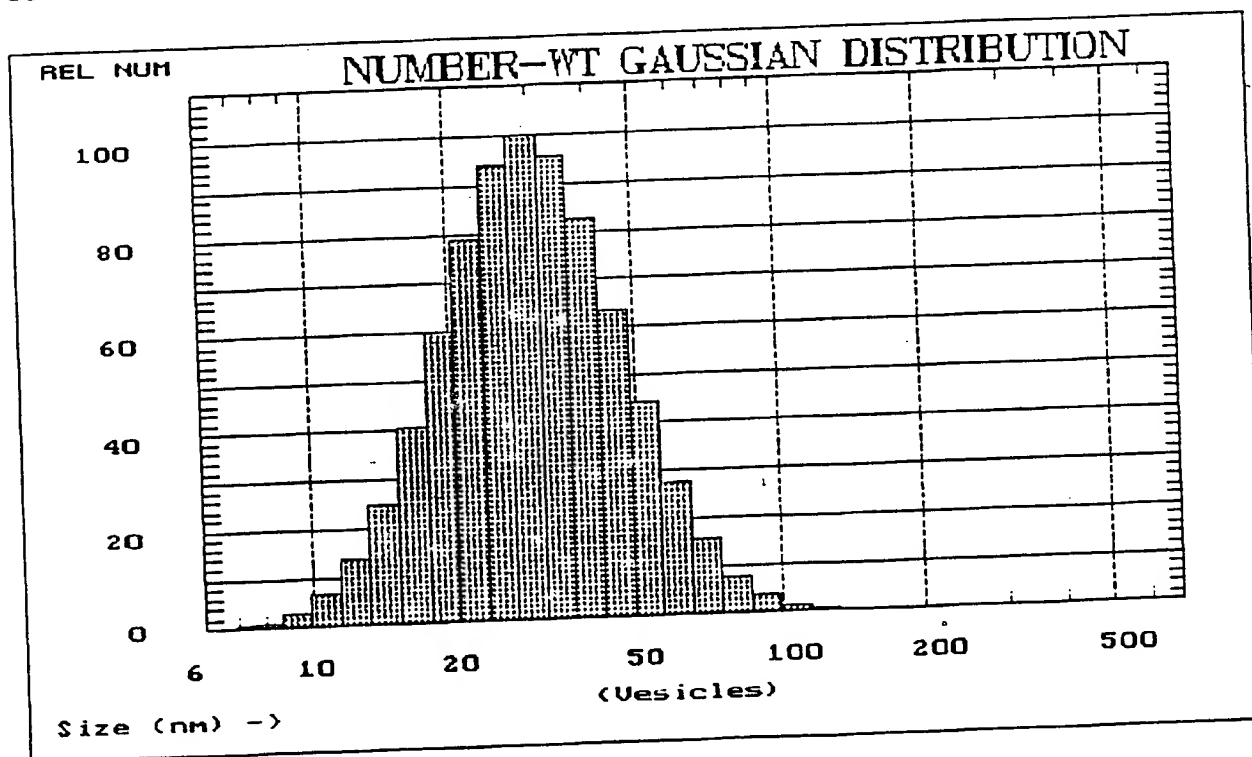
Std. Deviation = 14.4 nm (43.9 %)

Coeff. of Var'n = 0.439

Chi Squared = 0.347

Baseline Adj. = 0.000 %

Mean Diff. Coeff. = 1.42E-07 cm²/s



Cumulative Results:

25 % of distribution < 20.56 nm
50 % of distribution < 27.72 nm
75 % of distribution < 37.35 nm
90 % of distribution < 48.88 nm
99 % of distribution < 77.28 nm

Figure 16

Run Time = 1 Hr 43 Min 26 Sec
Count Rate = 303 KHz
Channel #1 = 2827.4 K
Channel Width = 8.0 uSec

Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

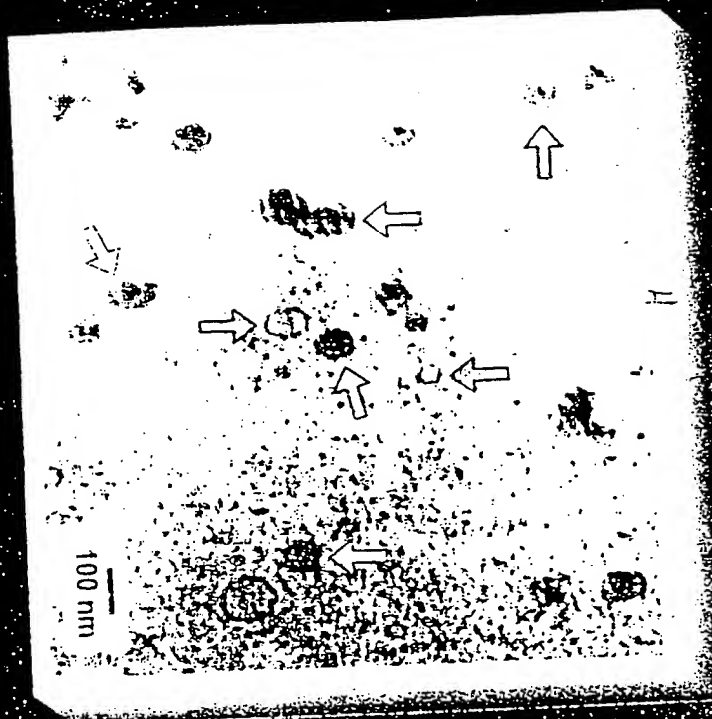


Figure 17A

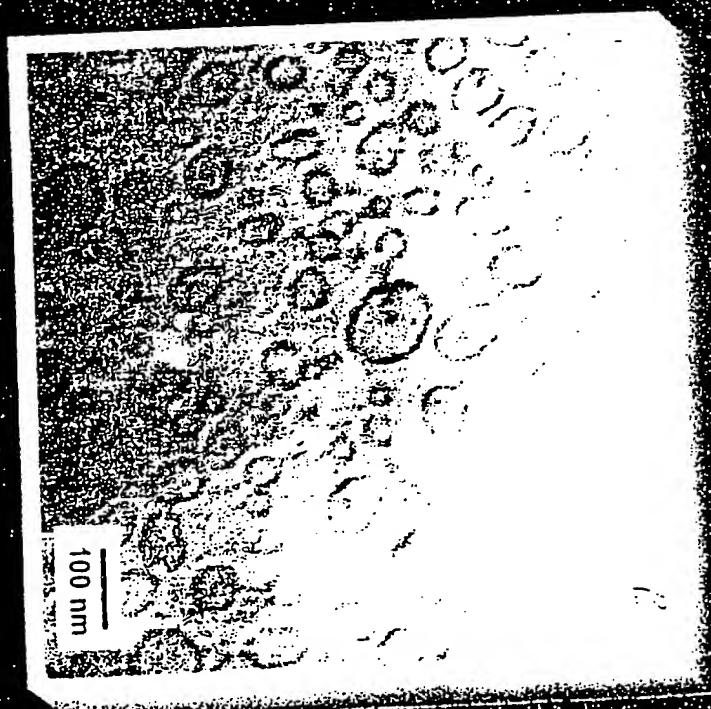


Figure 17B

PLASMID TCS

Clearance of DNA Encapsulated in POPC:DODAC:PEG-Cer(C20)

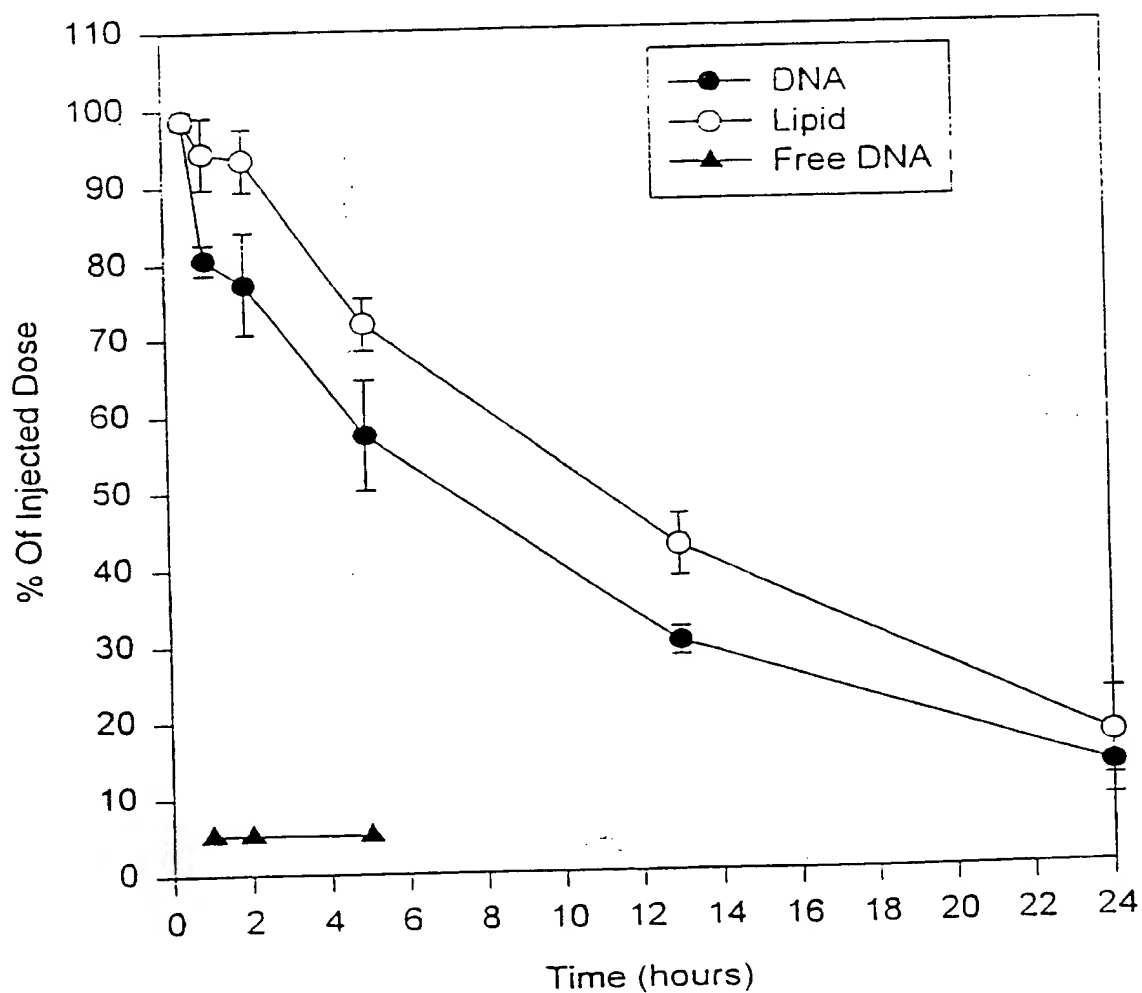


Figure 18

05/31/96

02:42

604 264 9959

INEX

040/043

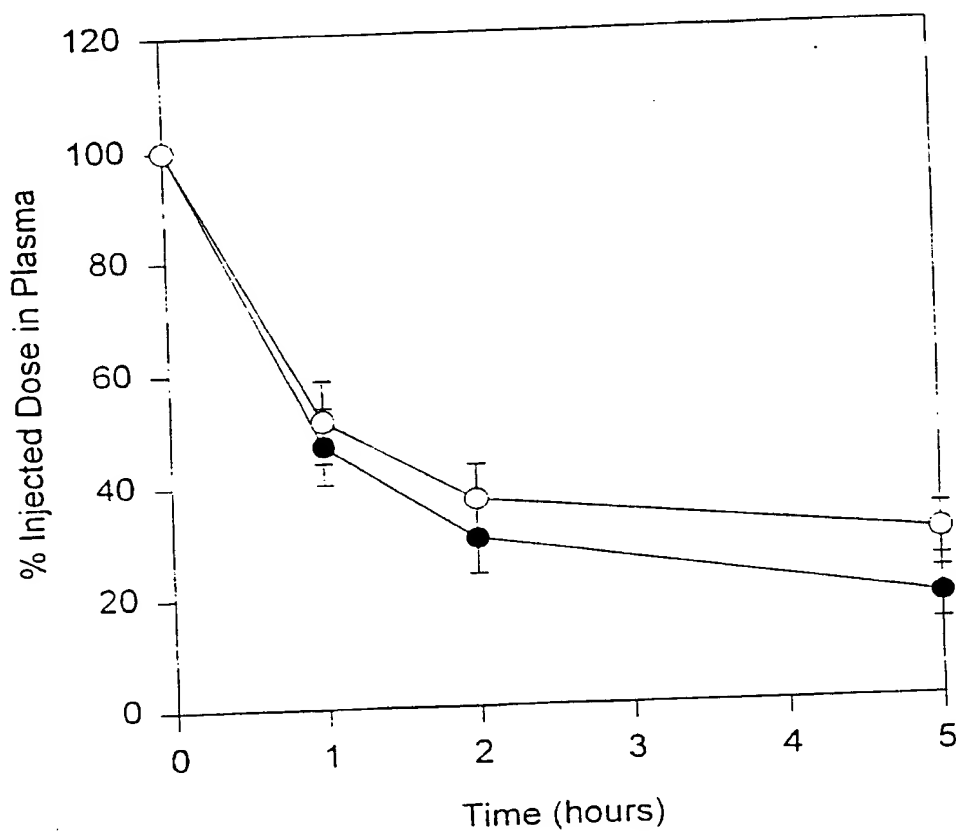


Figure 19A

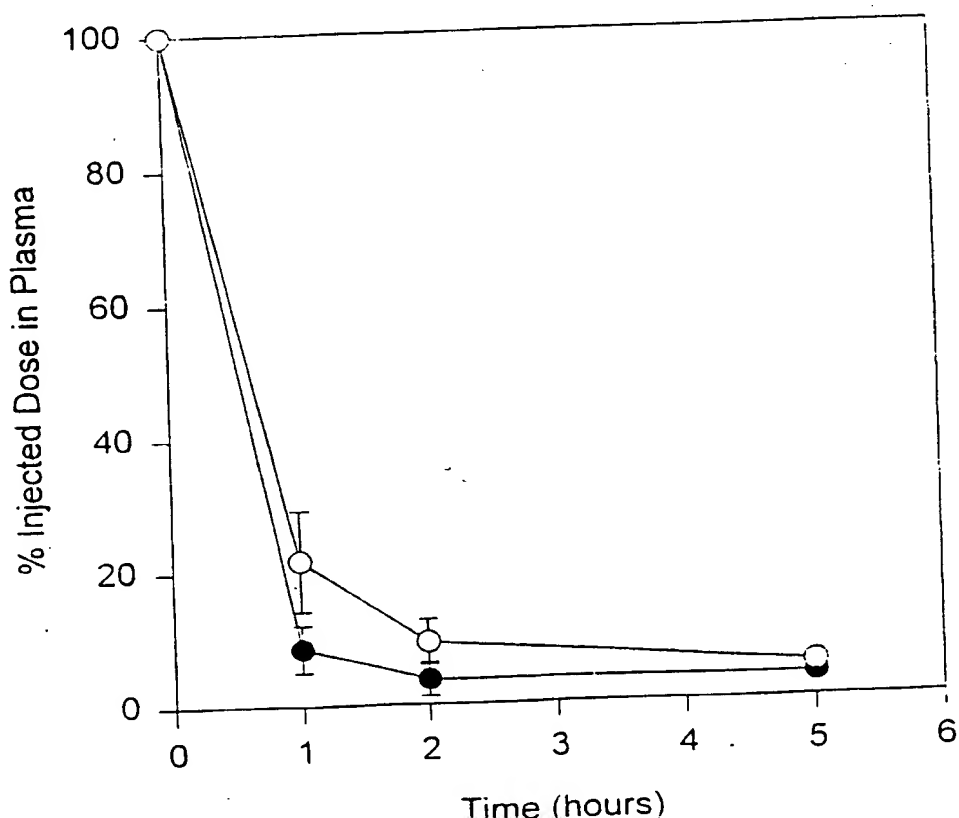


Figure 19B

In Vivo Transfection in the Lung

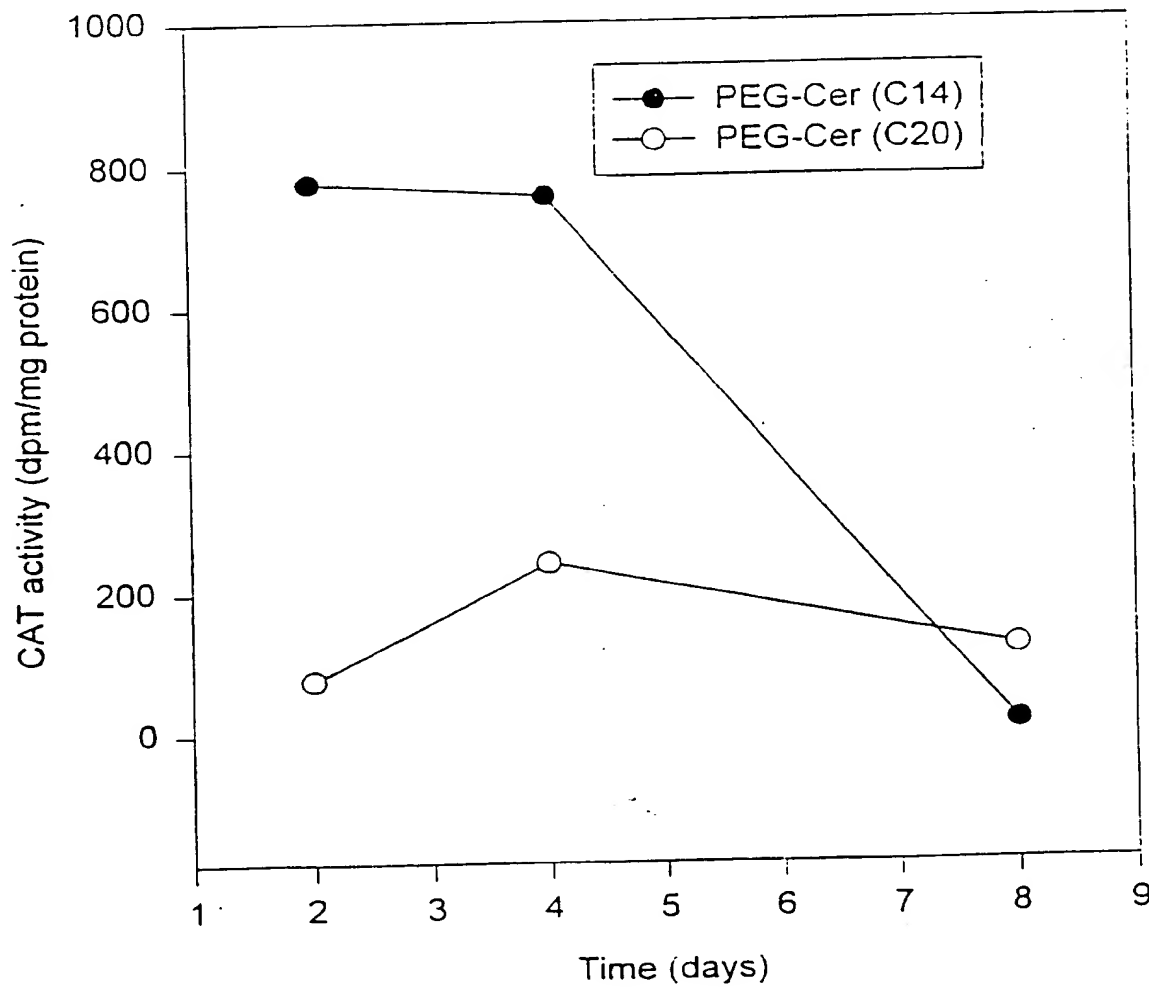


Figure 20

In Vivo Transfection in the Liver

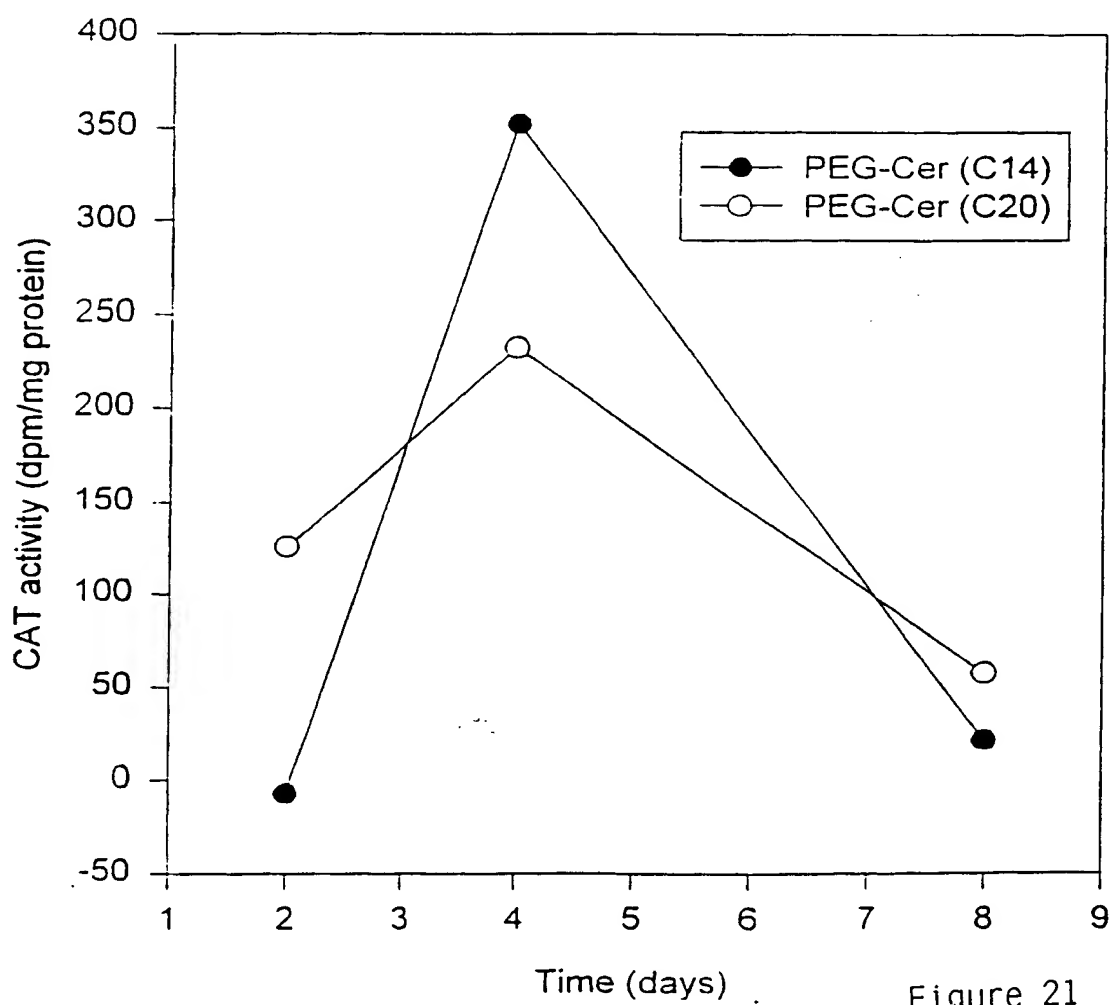


Figure 21

In Vivo Transfection in the Spleen

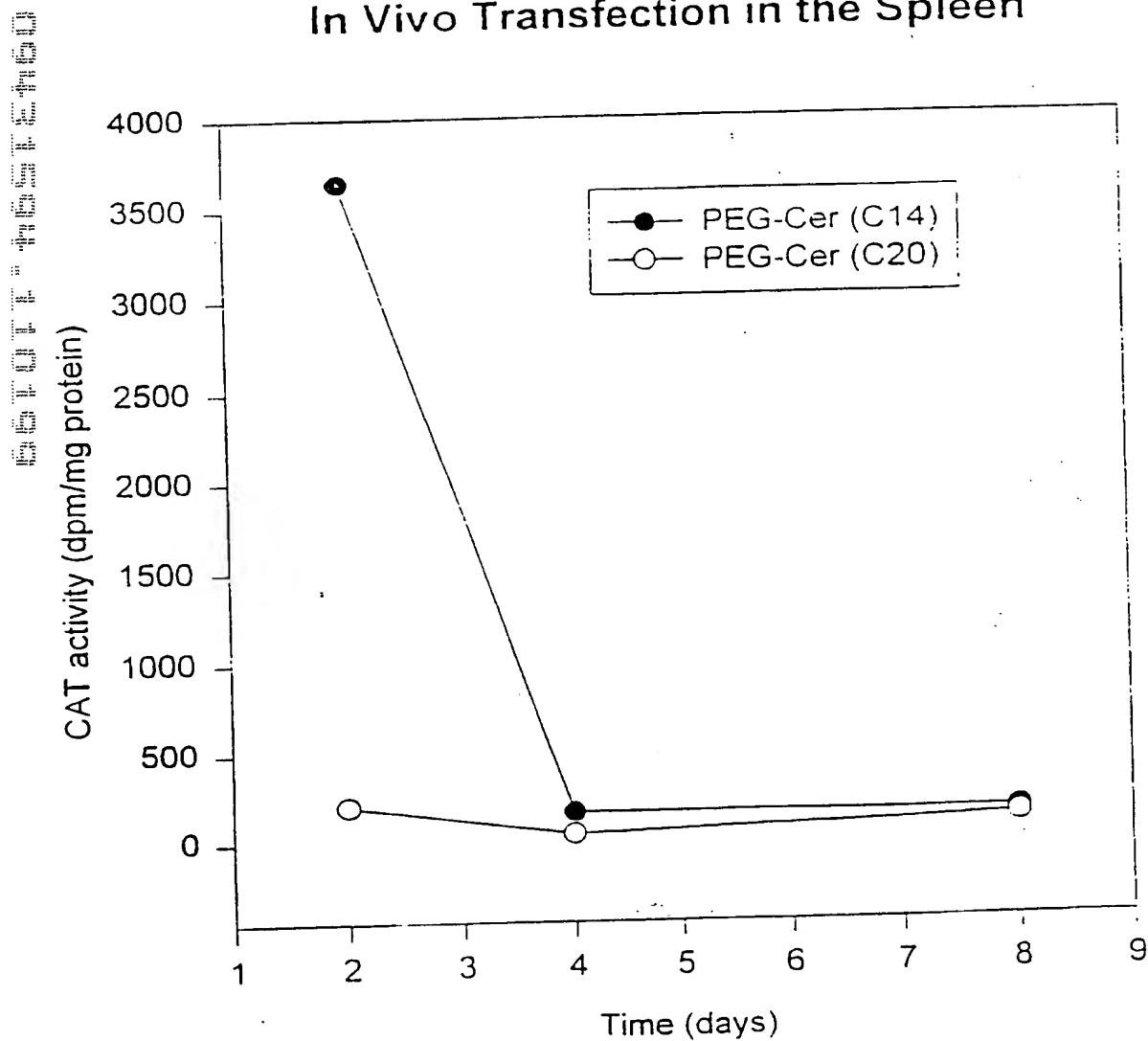


Figure 22

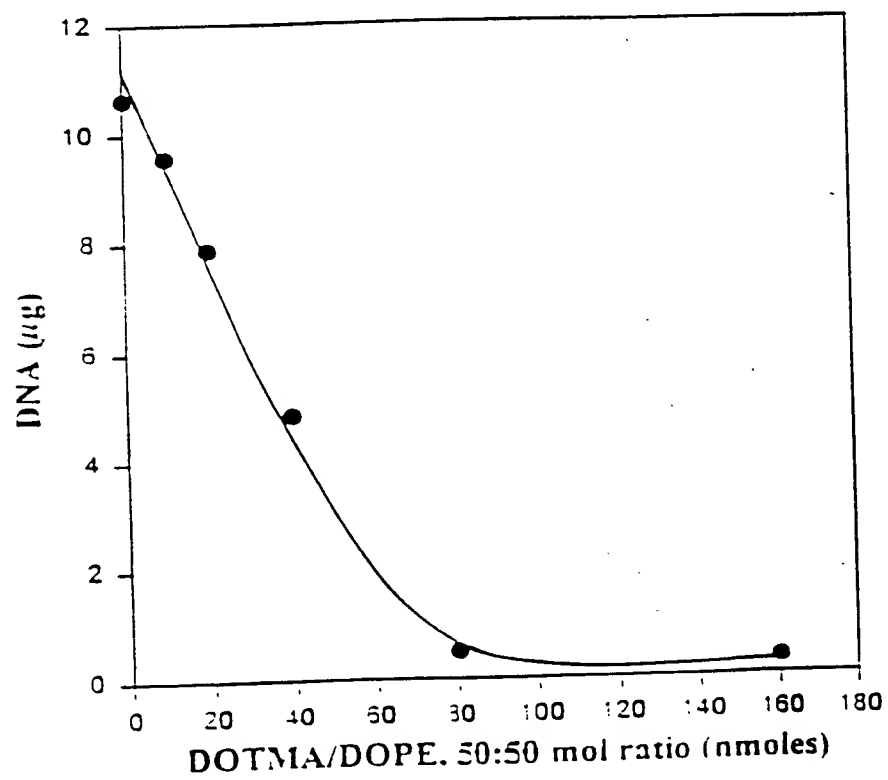


Figure 23

Figure 24A

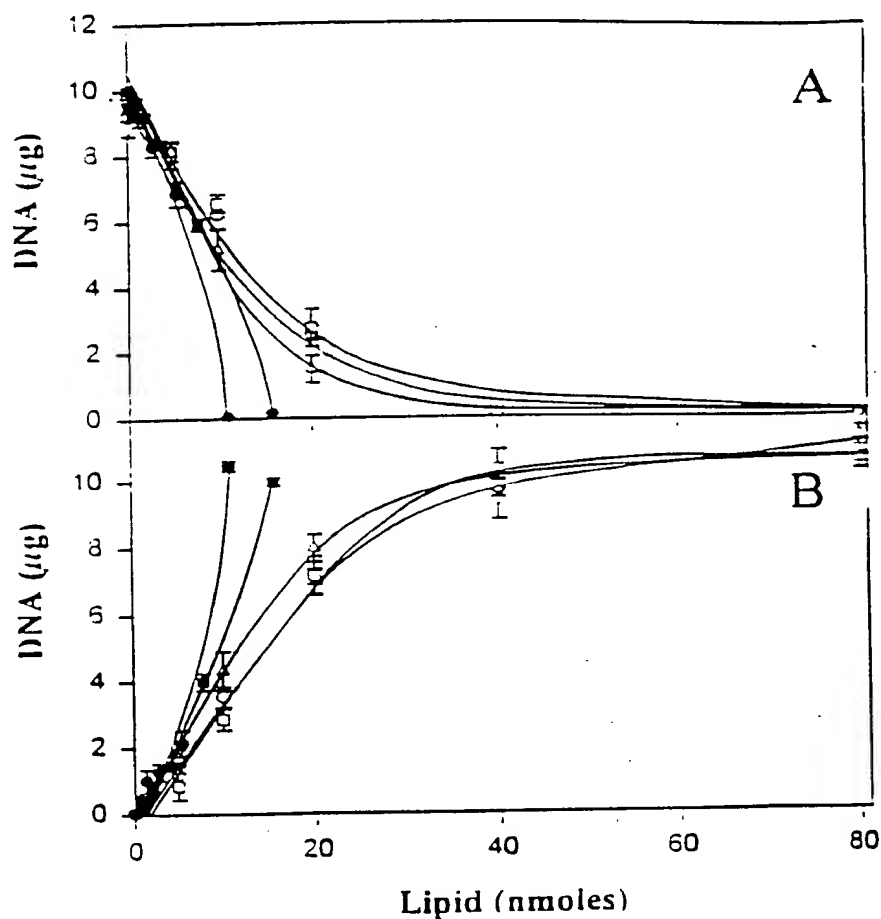


Figure 24B

Recovery of plasmid DNA in the aqueous (A) and solvent (B) phase following Bligh and Dyer extraction of the DNA/lipid complexes. DNA amount used was 10 μg . Monocationic lipids used were DDAB (O), DOTMA (\square) and DODAC (Δ). Lipopolyamines used were Lipofectamine (\bullet), and Transfectam (\blacksquare). All data points are averaged from three replications and expressed \pm SEM.

Figure 25A

Figure 25B

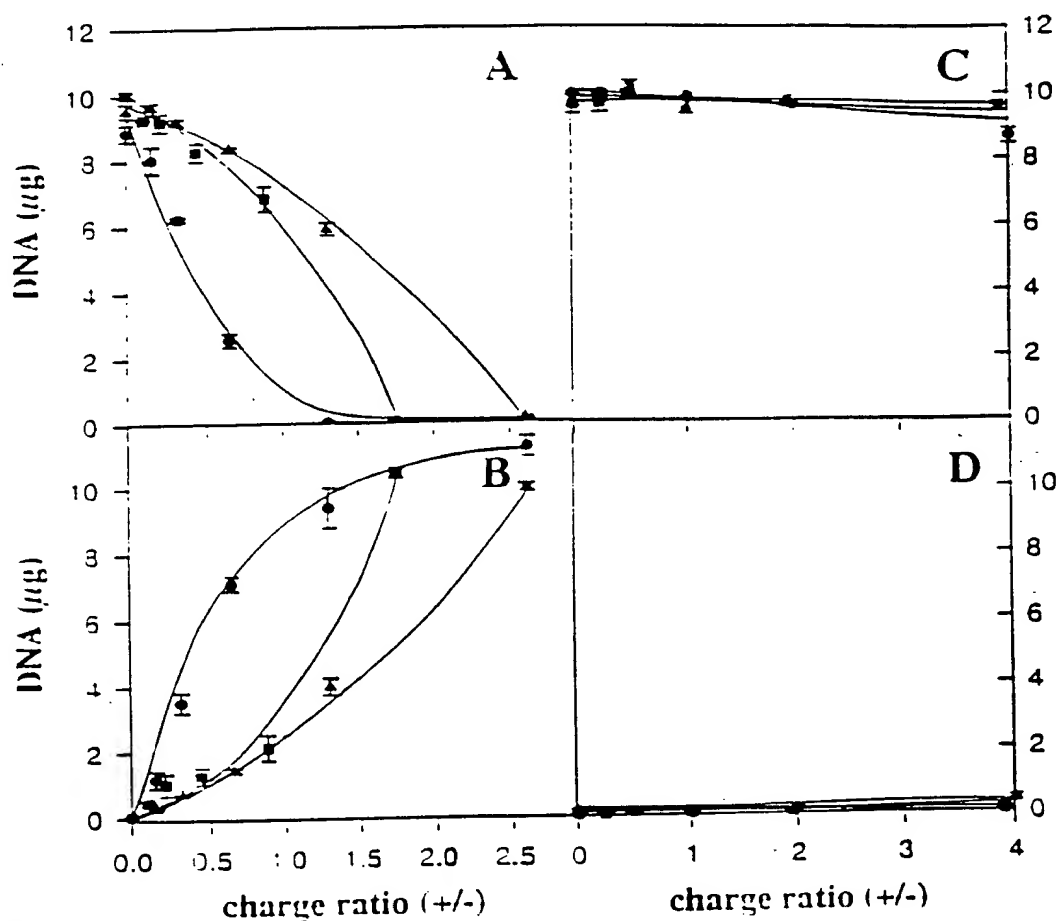


Figure 25C

Figure 25D

The recovery of plasmid DNA from aqueous (A and C) and solvent (B and D) fractions following Bligh and Dyer extractions and expressed as a function of charge ratio (+/-). (A and B), DDAB (●), Lipofectamine (■) and Transfectam (▲). (C and D), the effects of other cations, calcium (●), L-lysine (■), and poly-L-lysine (▲). DNA amount used was 10 μg and all data points were averaged from three experiments and presented \pm SEM.

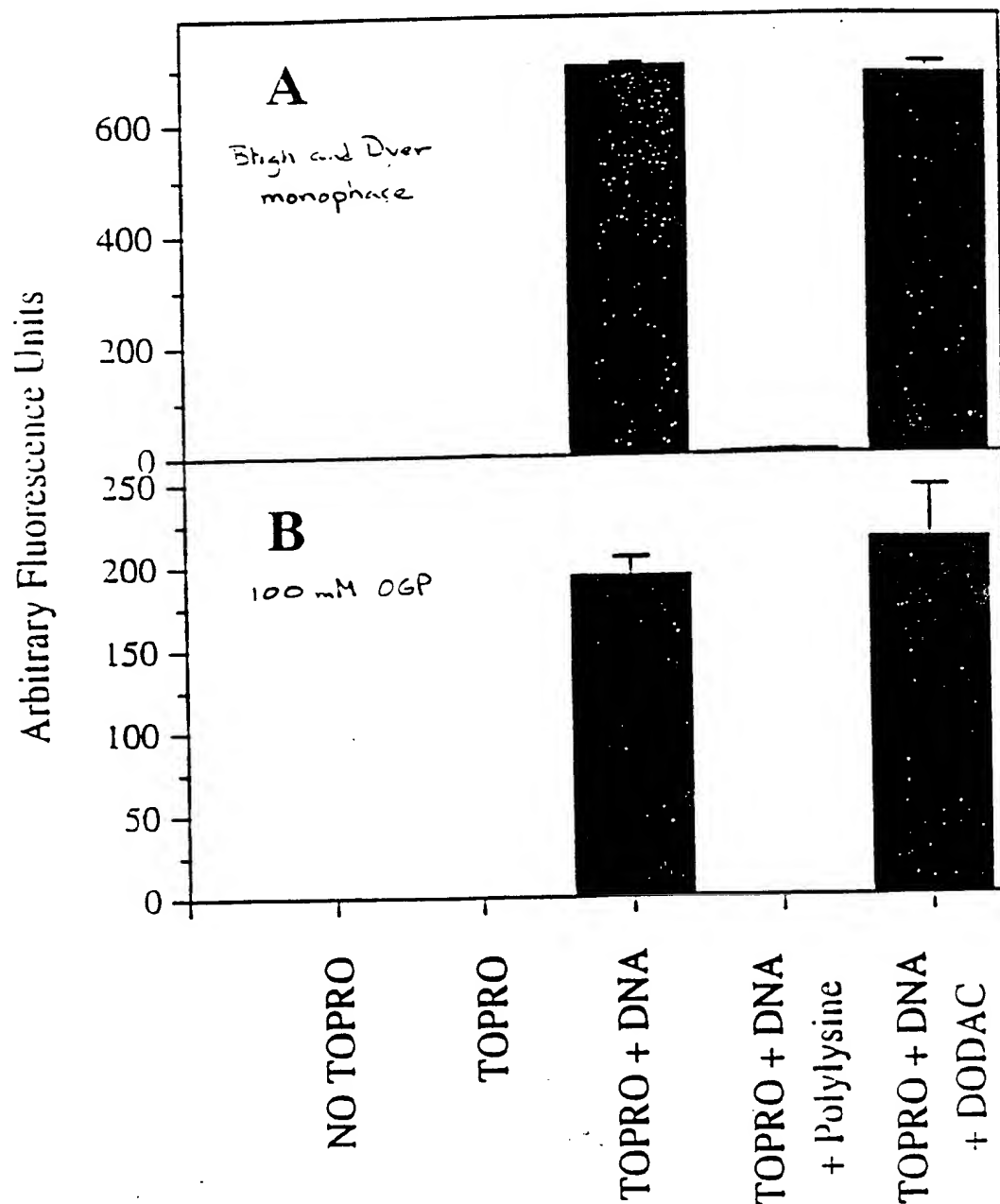
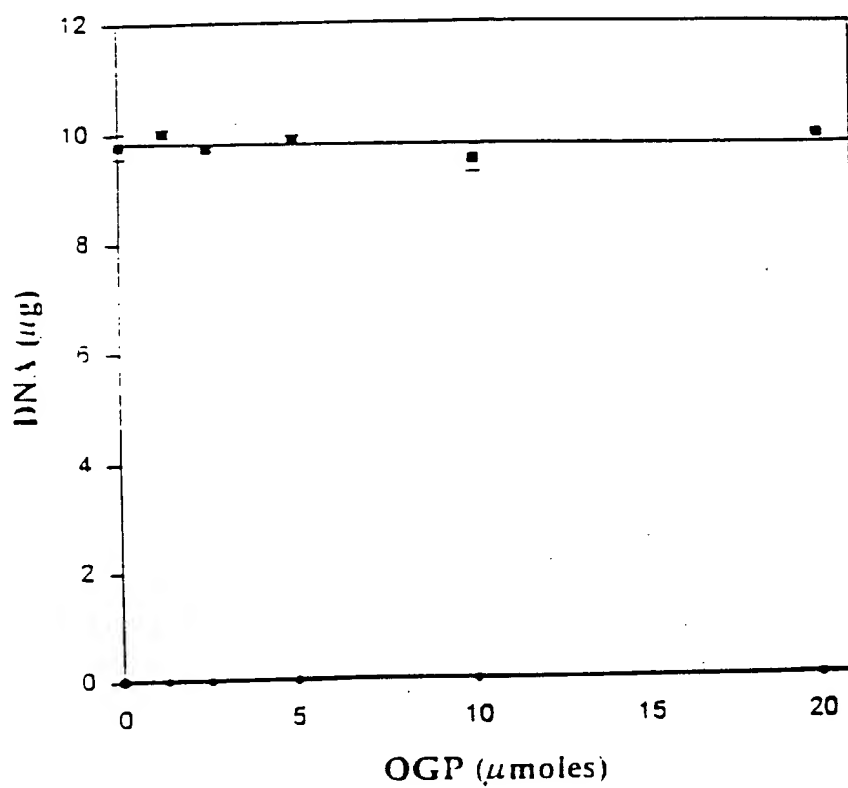


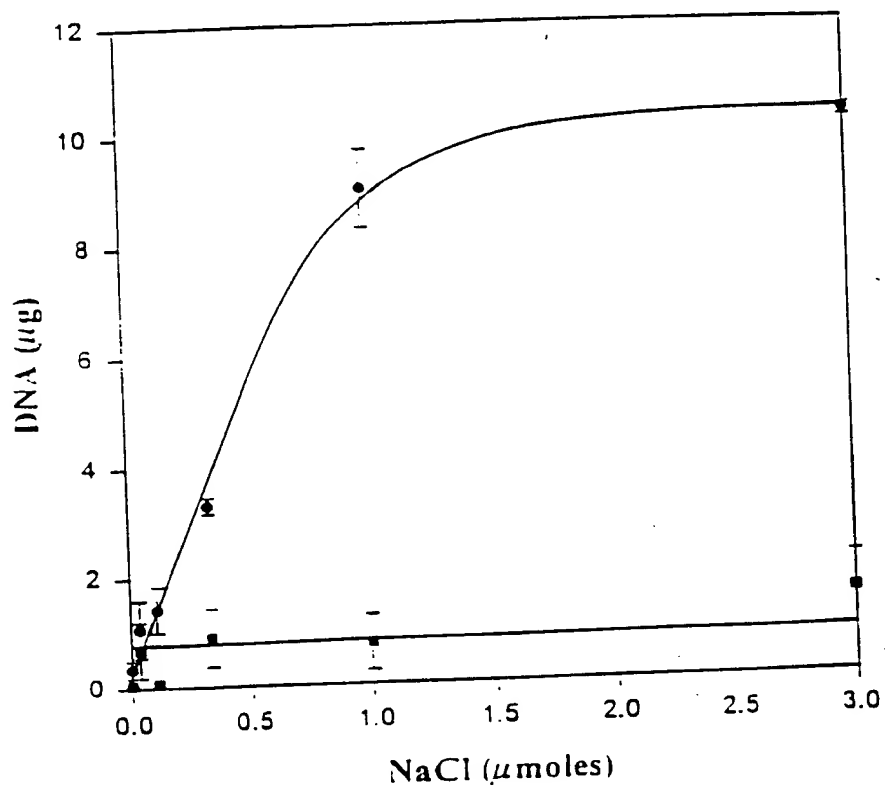
Figure 26A

Figure 26B



Effects of increasing amounts of OGP on the recovery of plasmid DNA from the aqueous (●) and solvent (■) phases following Bligh and Dyer extraction of

Figure 27



Effects of increasing amounts of NaCl on the recovery of plasmid DNA from the aqueous phase following Bligh and Dyer extraction of DNA/lipid complexes. Amount of DNA used was 10 μ g. DODAC (●), Lipofectamine (■).

Figure 28

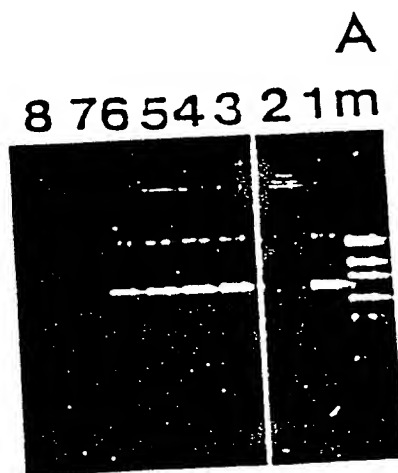


Figure 29A

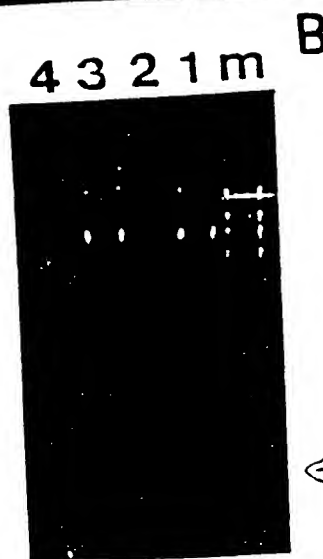


Figure 29B



551011-1651E450

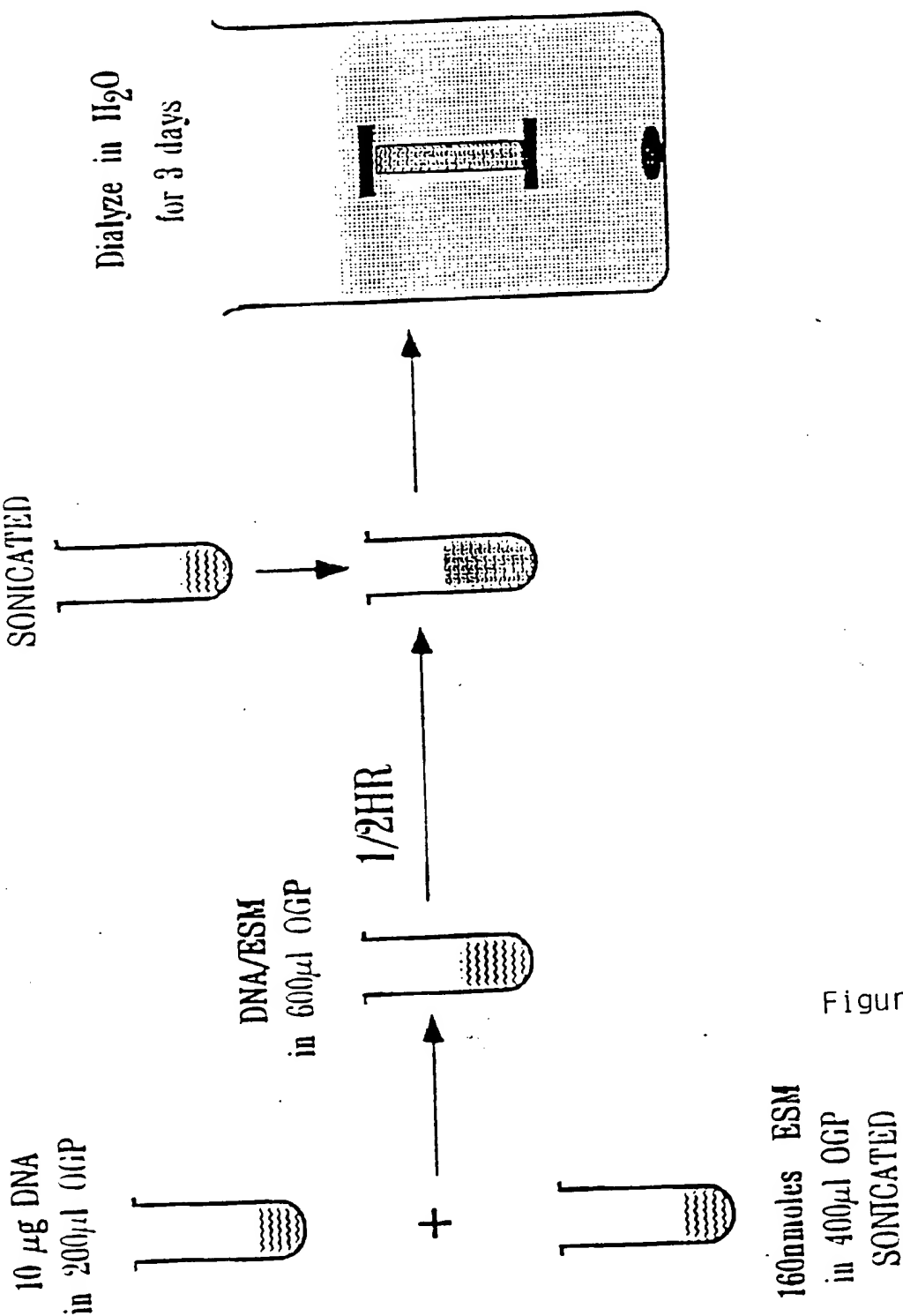


Figure 30

20 μ g β -gal DNA/160nmole DODAC/320nmole ESM

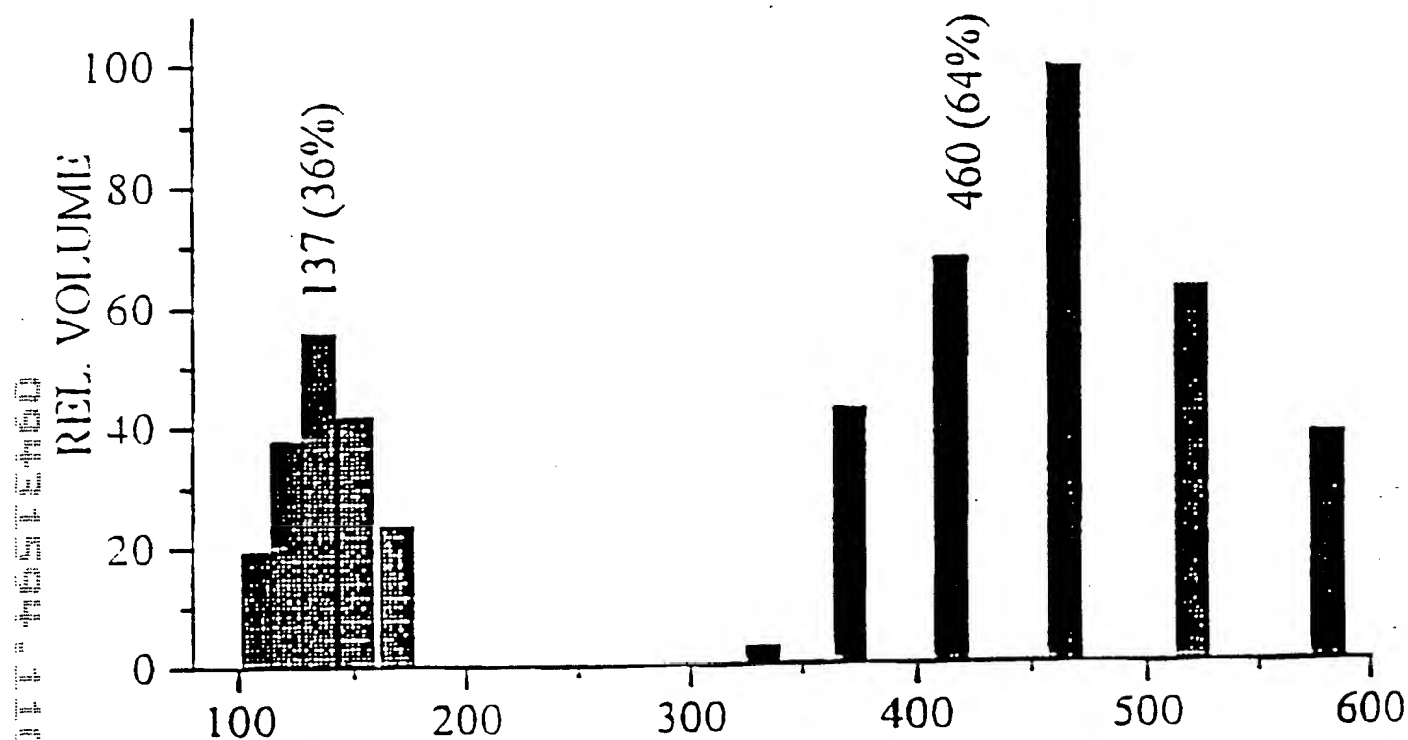


Figure 31A

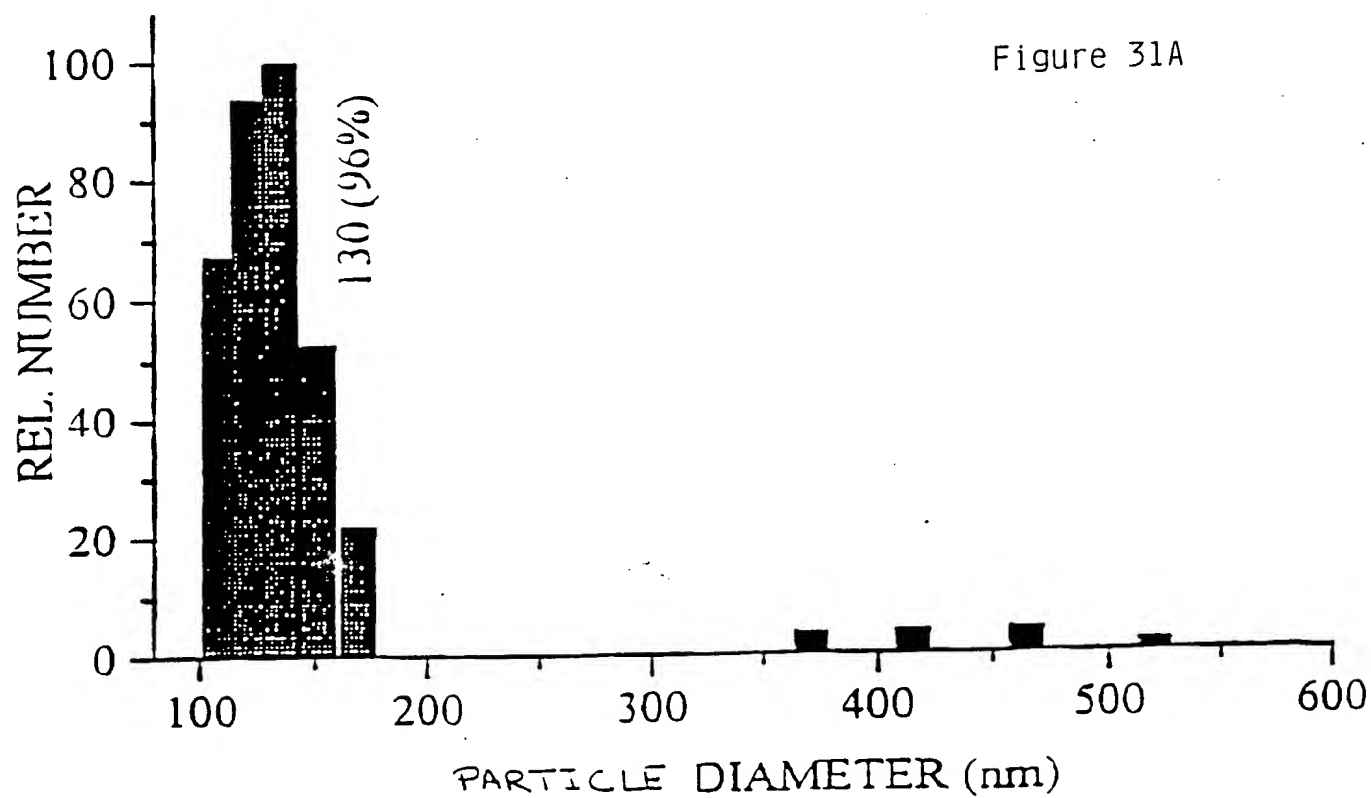
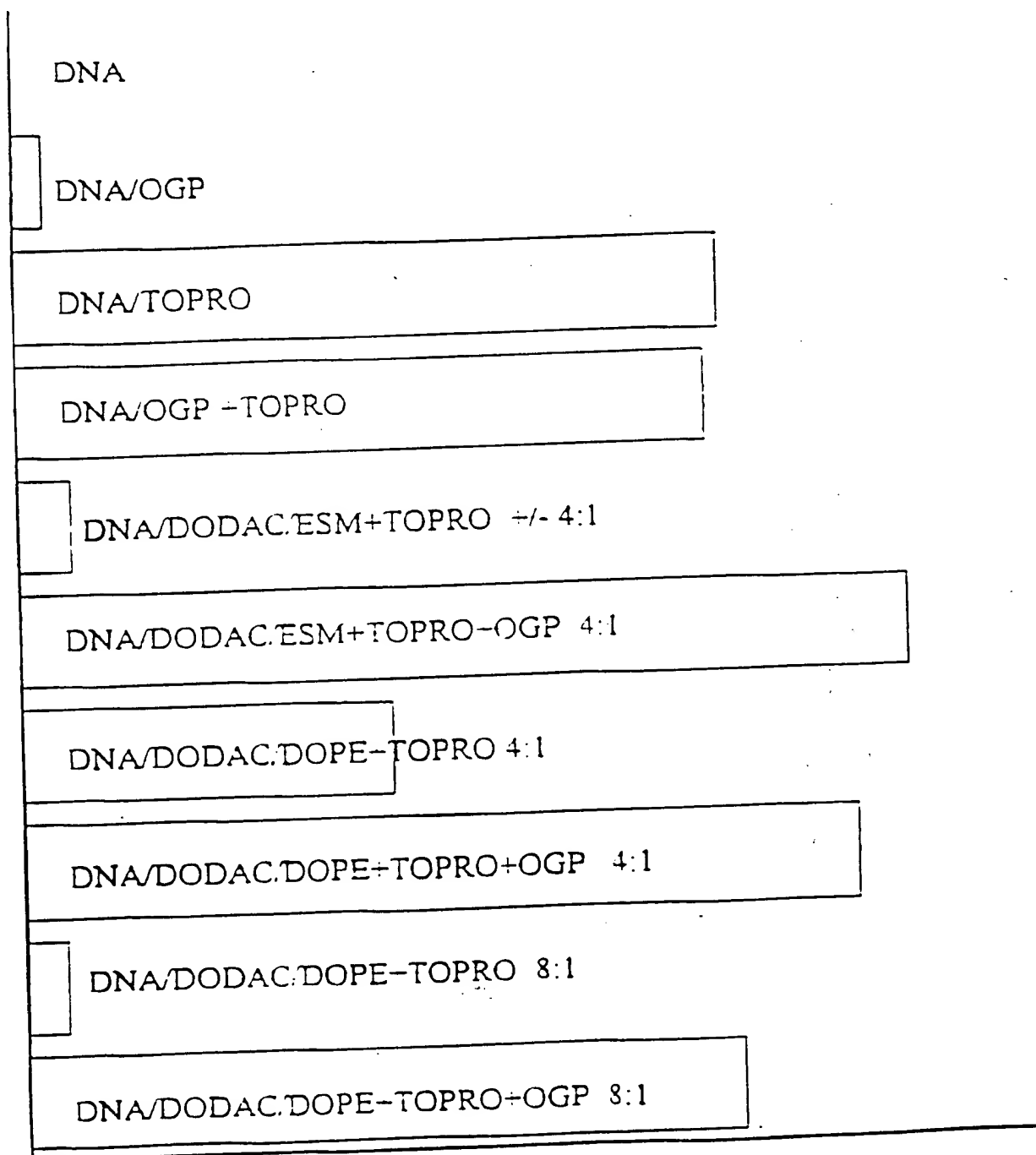


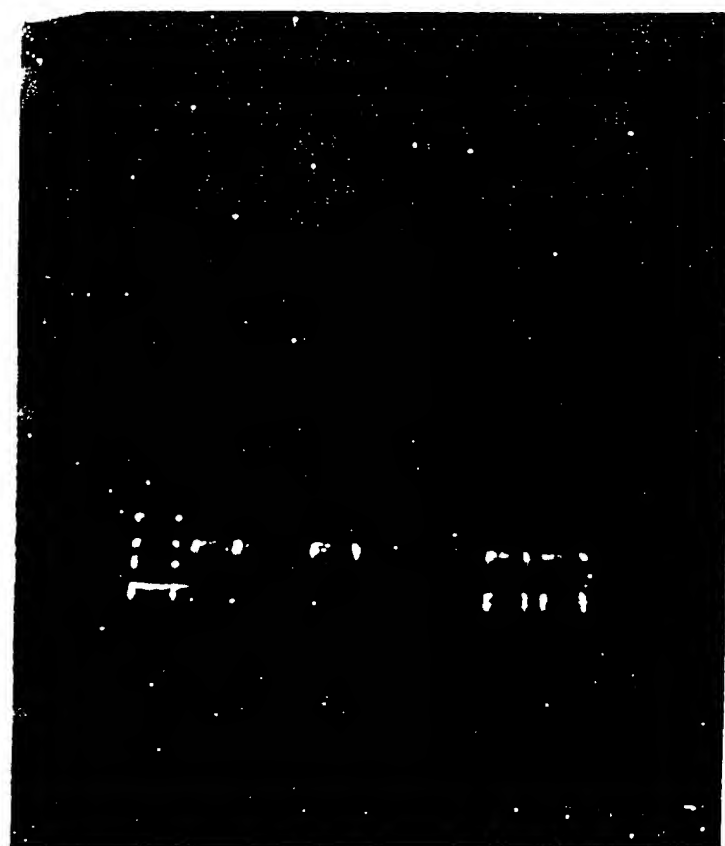
Figure 31B



FLUORESCENCE INTENSITY

Figure 32

09431594-110195



M 3
DNA
+DNase
complex
+DNase
LAP + DNase
LAP + DNase

Figure 33

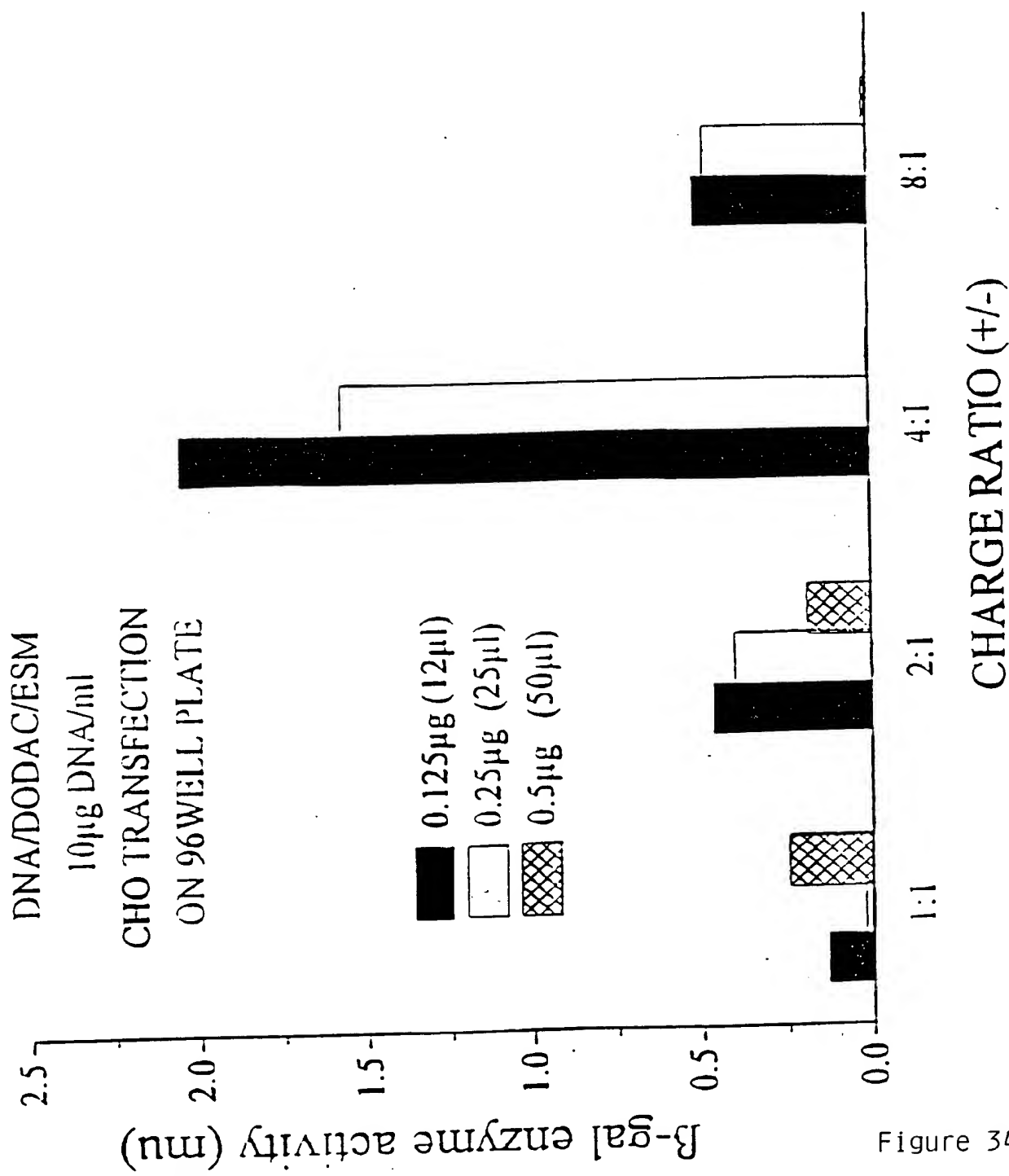


Figure 34

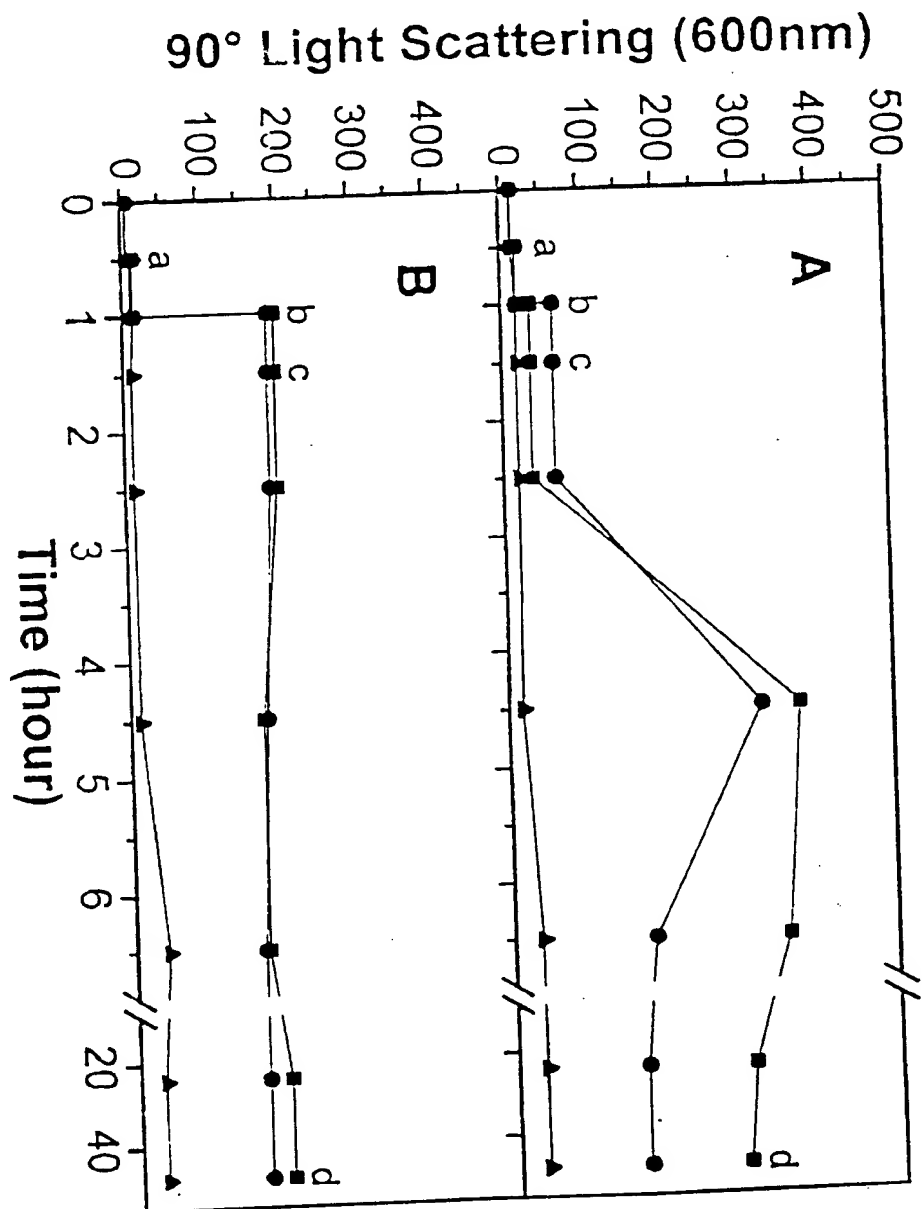


Figure 35A

Figure 35B

09431594-110199

90° Light Scattering (600nm)

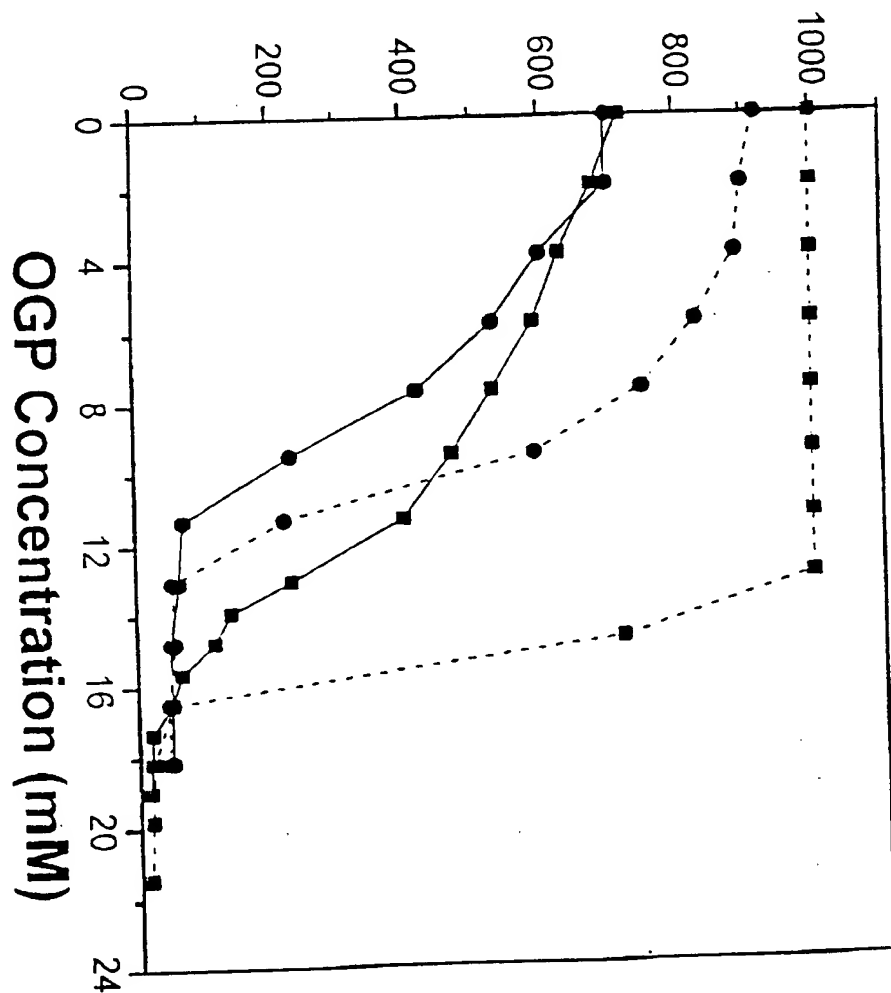


Figure 36

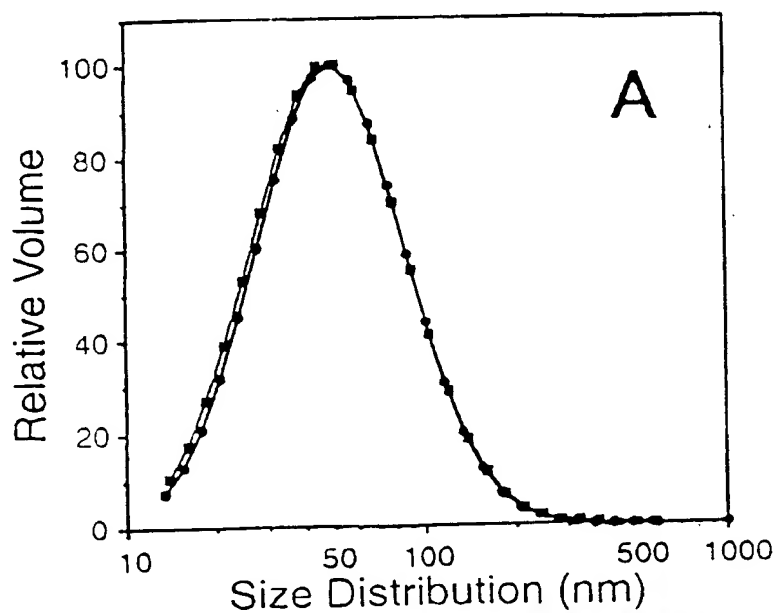


Figure 37A

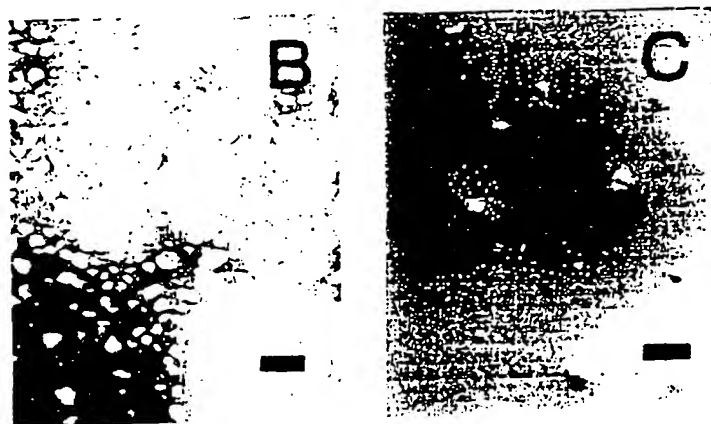


Figure 37B

Figure 37C

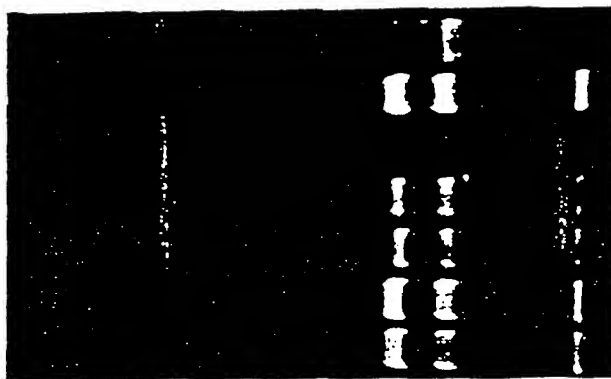
Figure 38B

B
1 2 3 4 5



Figure 38A

A
1 2 3 4 5 6 7



00431594-110199

β -galactosidase activity (mU/well)

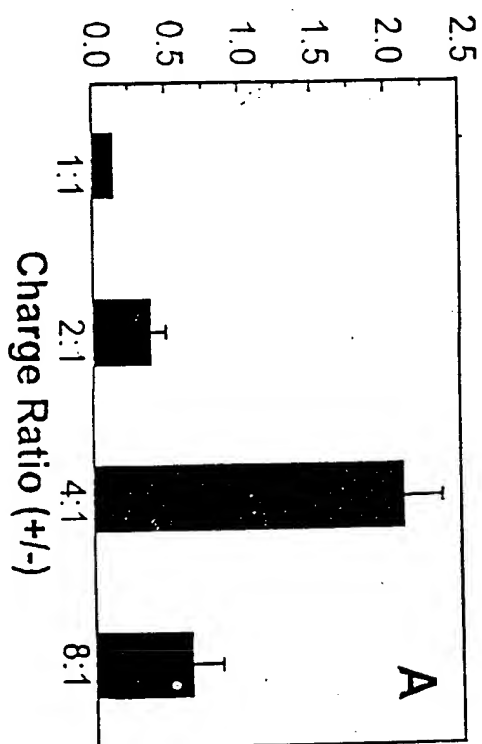


Figure 39A

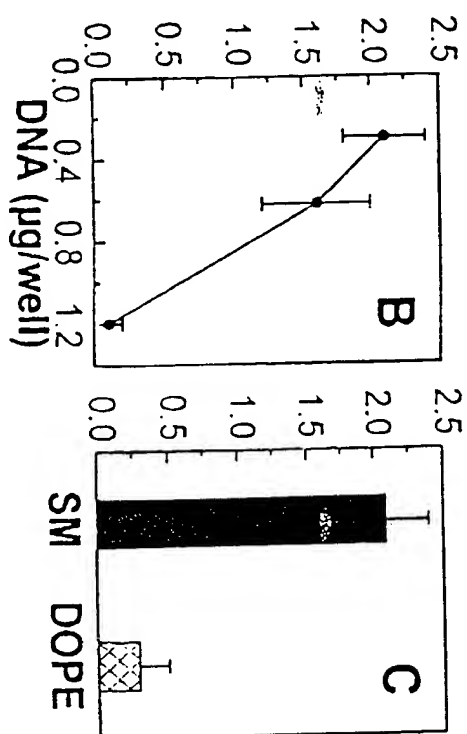
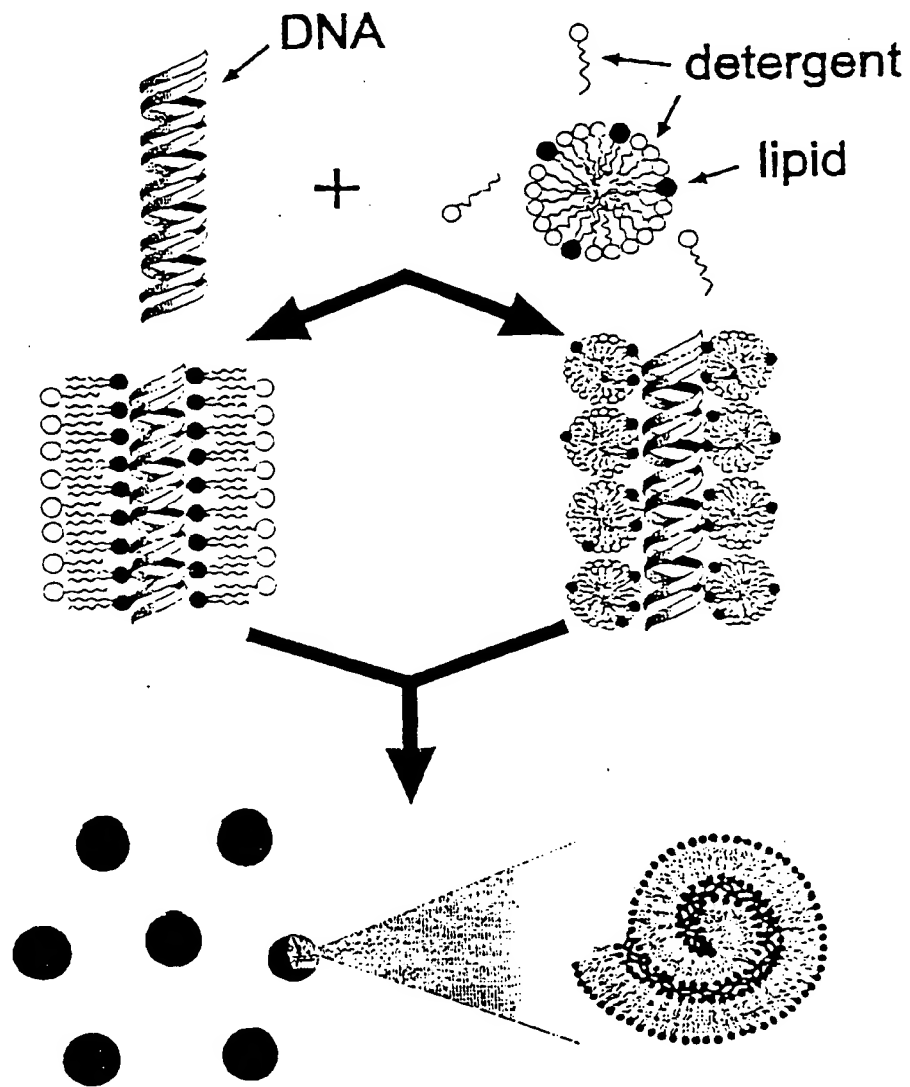


Figure 39C

Figure 39B



DNA-Lipid Particle Formation

Figure 40

% Recovery of pINEXCAT with different composition of cationic lipid/DOPE/10 mol % PEG-CER C14 (5.0 μ mol total lipid) from DEAE Sepharose CL6B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

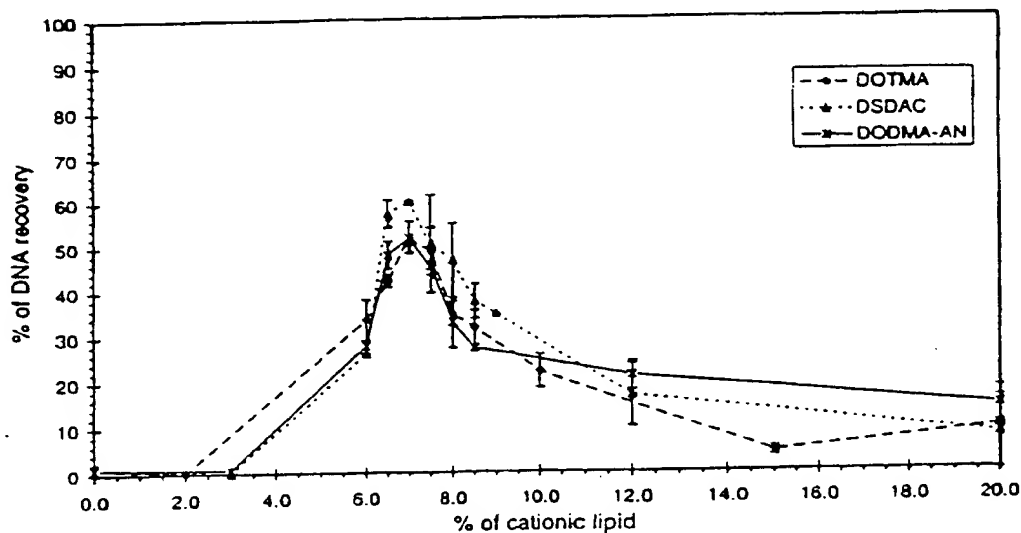


Figure 41A

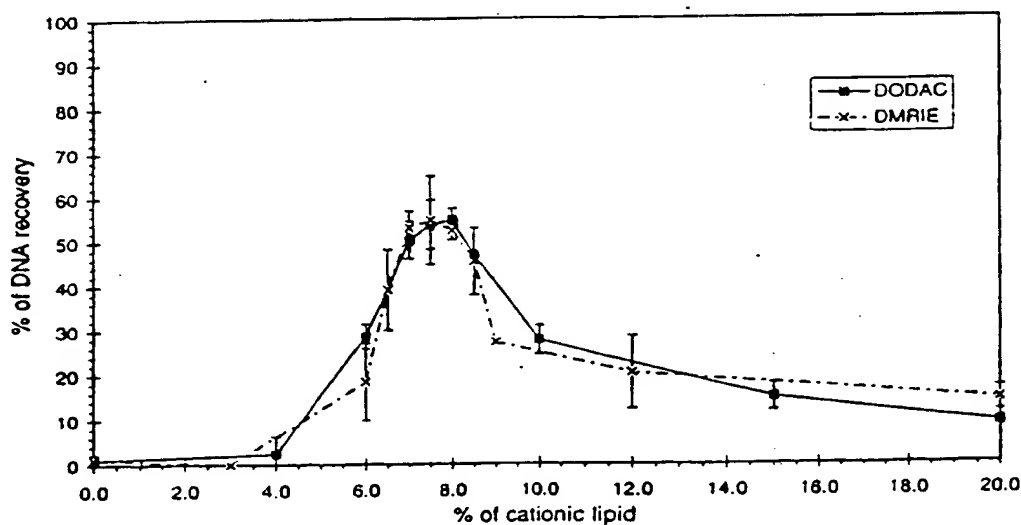


Figure 41B

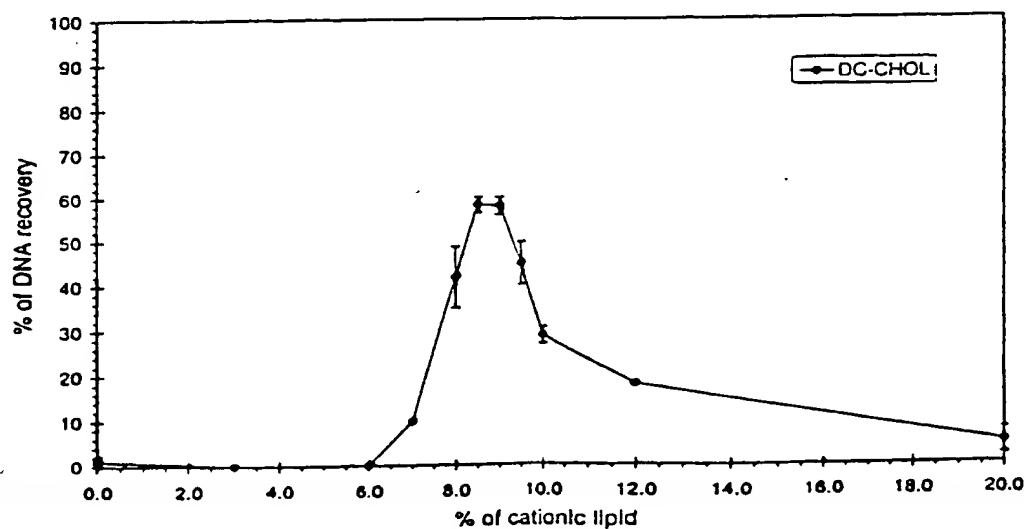


Figure 41C

Elution profile of 7.0 mol % DODAC/ 83 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

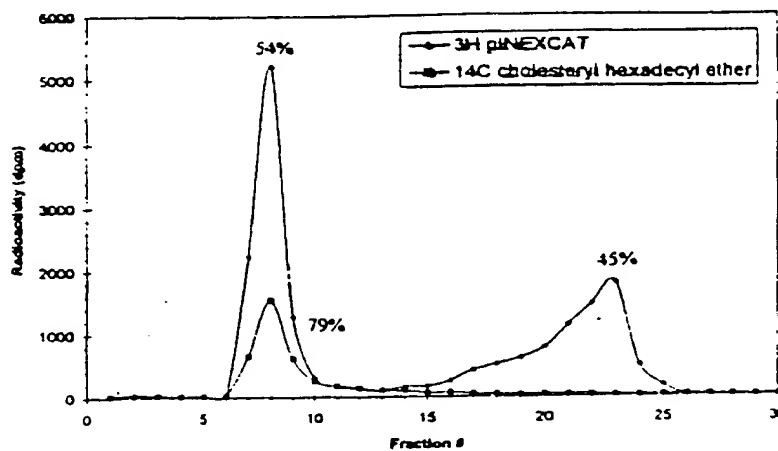


Figure 42A

(a)

Elution profile of 7.0 mol % DOTMA/ 83 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

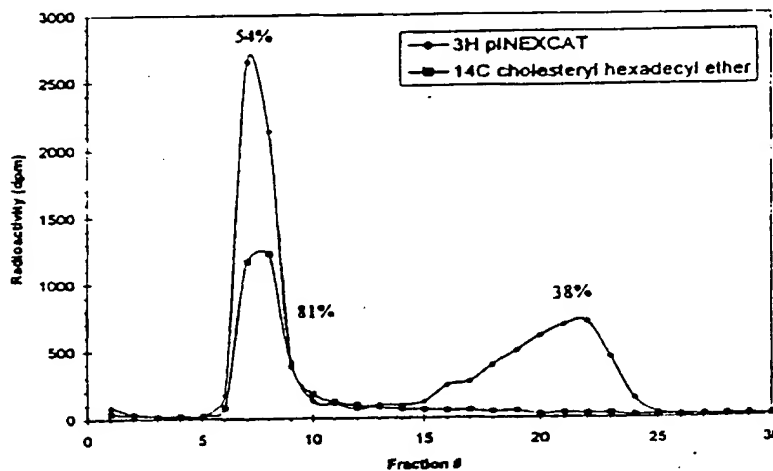


Figure 42B

(b)

Elution profile of 7.5 mol % DSDAC/ 82.5 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

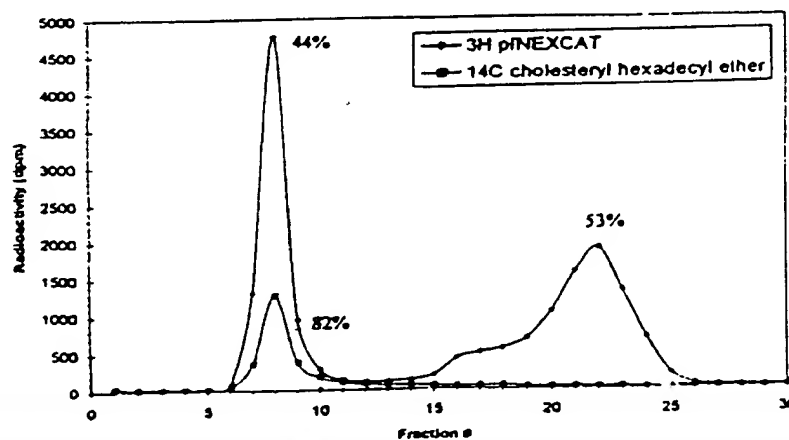


Figure 42C

(c)

EXAMPLE C

**Cationic Lipid Titration of 50 μ g pCMV β in POPC:DOPE:PEG-CerC8:AL-1
(65-x:25:10:x) Liposomes As Analyzed by the PicoGreen Assay**

Encapsulation Performed at pH4.8 or pH7.5

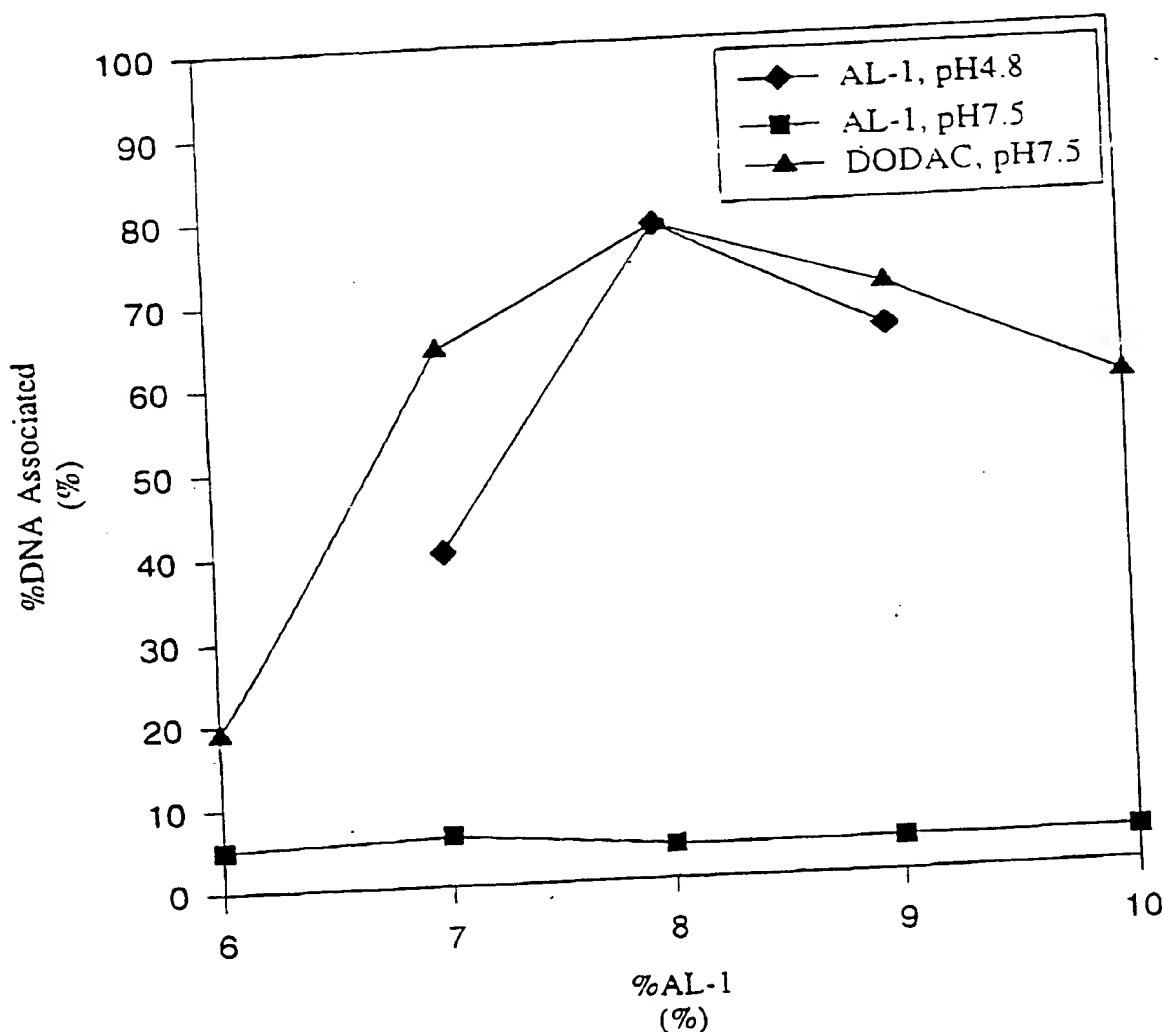


Figure 43

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06/04/96 TUE 16:08 FAX 604 822 4343

INEX
LIPOSM RES UNIT

007/009
003

EXAMPLE D

(a)

Serum Stability (1.5 hr at 37°C) of 50µg pCMVβ
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)
Liposomes {pH4.8 Encapsulation}

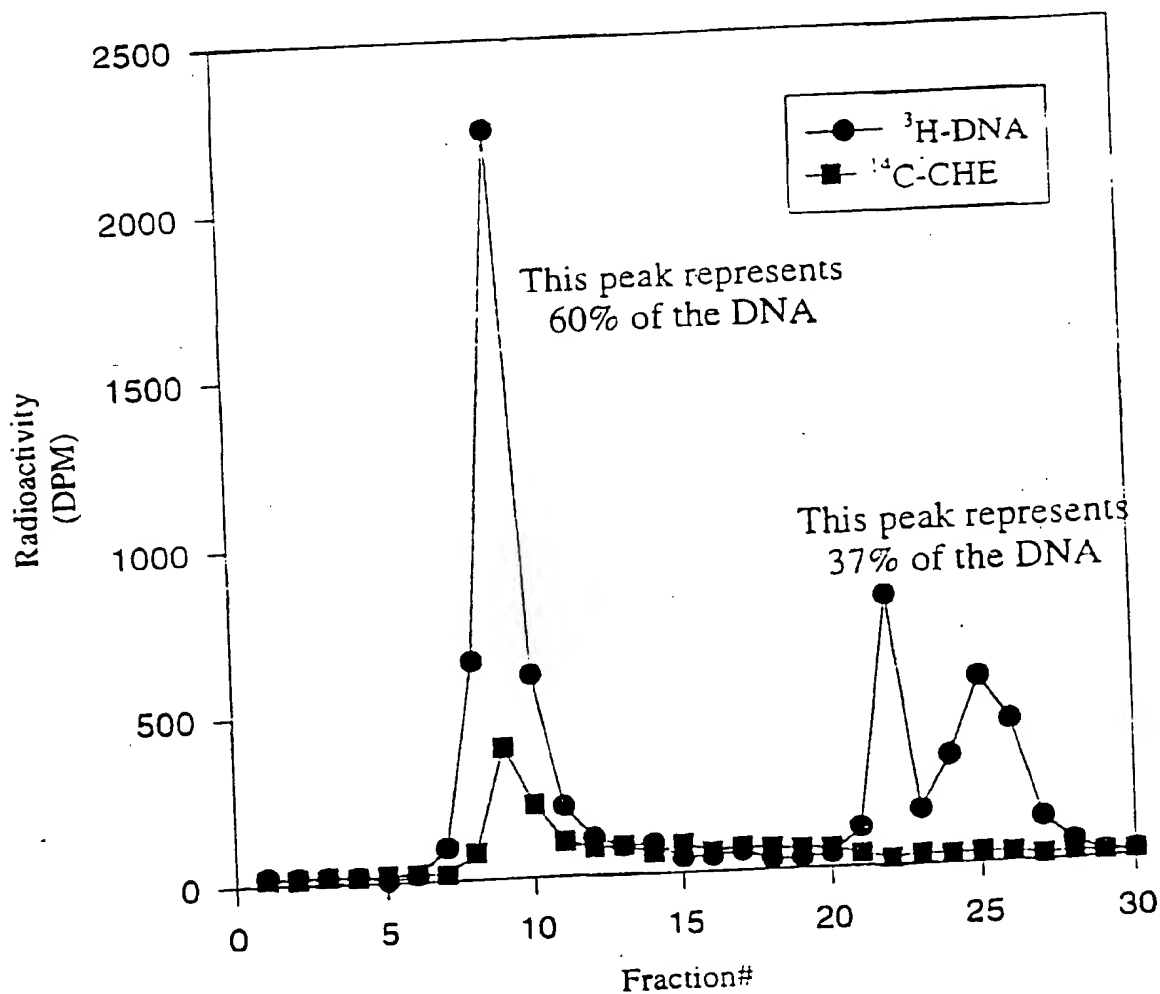


Figure 44

06/05/96 13:41 604 264 9959
06/04/96 TUE 16:03 FAX 604 922 4843

INEX
LIPOSM RES UNIT

008/009
004

EXAMPLE D
(b)

Serum Stability (1.5 hr at 37°C) of 50µg pCMVβ
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)
Liposomes {pH7.5 Encapsulation}

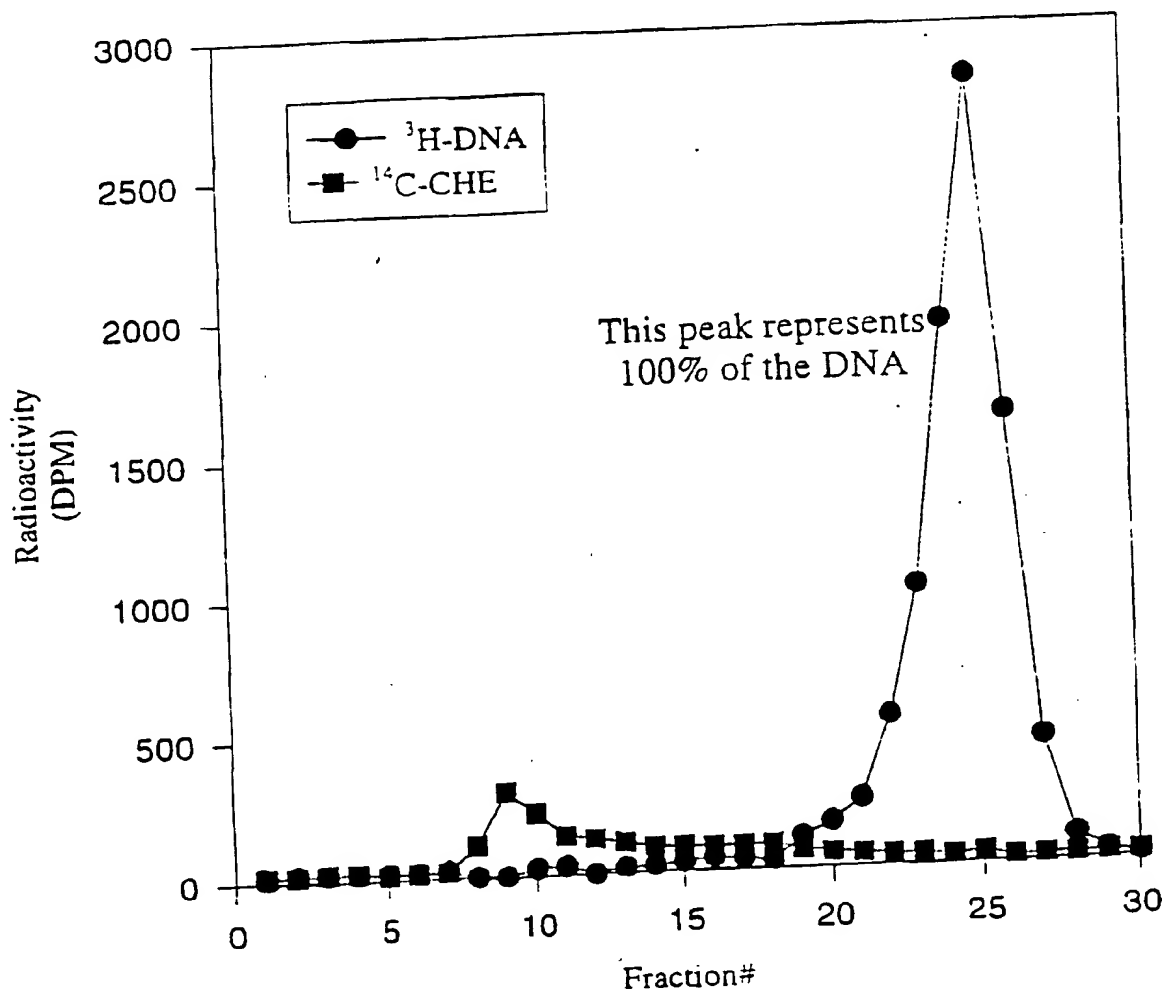


Figure 45

EXAMPLE E

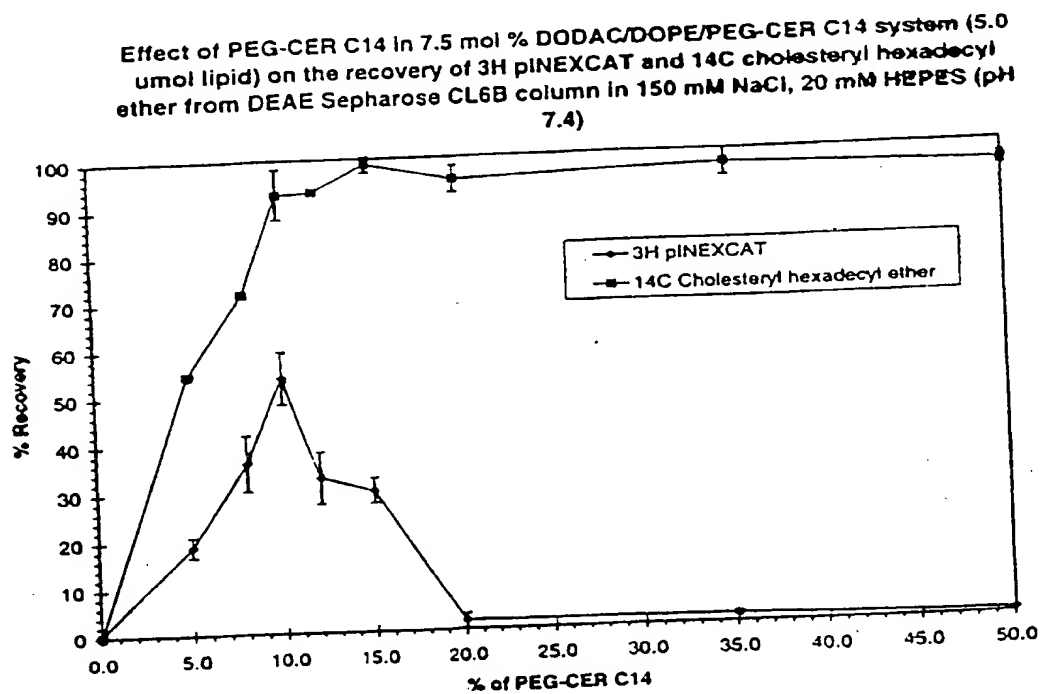


Figure 46